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A Study of Existing Cross-Site Scripting Detection and Prevention Techniques Using XAMPP and VirtualBox

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ABSTRACT

Most operating websites experience a cyber-attack at some point. Cross-site Scripting (XSS) attacks are cited as the top website risk. More than 60 percent of web applications are vulnerable to them, and they ultimately are responsible for over 30 percent of all web application attacks. XSS attacks are complicated, and they often are used in conjunction with social engineering techniques to cause even more damage. Although prevention techniques exist, hackers still find points of vulnerability to launch their attacks. This project explored what XSS attacks are, examples of popular attacks, and ways to detect and prevent them. Using knowledge gained and lessons-learned from analyzing prior XSS incidents, a simulation environment was built using XAMPP and VirtualBox. Four typical XSS attacks were launched in this virtual environment, and their potential to cause significant damage was measured and compared using the Common Vulnerability Scoring System (CVSS) Calculator. Recommendations are offered for approaches to impeding XSS attacks including solutions involving sanitizing data, whitelisting data, implementing a content security policy and statistical analysis tools.

Keywords: Cross-Site Scripting (XSS) Attack, XAMPP, VirtualBox

1. INTRODUCTION

Today, millions of users rely on web applications for bank information, education, and social media. However, the presence of security vulnerabilities creates risk when they use these applications. Malicious users can take advantage of these vulnerability to steal sensitive information, send illegal Hypertext Transfer Protocol (HTTP) requests, redirect unsuspecting users to harmful websites, install malware, and perform other malicious operations (Gupta, Govil, & Singh, 2015). XSS is a common cyber-attack typically found in web applications (Cross-site scripting). XSS attacks are a type of injection that occurs when hackers exploit a weakness in an otherwise benign and trusted webpage to insert their own malicious code. That code can be implemented to steal users' personally identifiable

information (PII) such as credentials, session cookies, sensitive data; and it even can live persistently on a site to continue attacking multiple users (Vigliarolo, 2018).

XSS attacks can cause significant damage to individuals, businesses, and other enterprises. According to a Ponemon Institute study on the *Cost of a Data Breach*, the mean time to identify a cyber-attack is 197 days, and the average total cost per breach is US$3.86 million (IBM, 2018). XSS attacks can negatively impact a company's reputation too, which leads to loss of productivity and revenue. XSS attacks have targeted social networks such as MySpace, Orkut, LinkedIn, Twitter and Facebook, exposing hundreds of millions of users to potential PII theft and other nefarious actions.

There are two main approaches to inserting malicious code into a webpage: reflected-XSS and stored-XSS attacks (Vogt, 2006). There is a third, less well-known type of XSS attack called DOM-based XSS that is beyond the scope of this paper. A reflected-XSS attack is delivered to the victim by an indirect means, such as an e-mail message or another website. The user may be tricked into clicking on a link, submitting a form, or even just browsing to a website. This provides an opportunity for the hacker to send malicious code as part of a server request that travels to a vulnerable website, then back to the user's browser. The user's browser executes the code because it appears to be coming from a trustworthy source.

A stored-XSS attack involves malicious content stored on the target server. If a user requests stored information from that server, such as from a webpage that contains a malicious script, the code is returned as part of the message. For example, an attack executed in the victim's web browser might transfer cookies to a web server that is controlled by the attacker. Cookies are the easiest way to locate and verify users and are used on most web browsers. This makes them an attractive target for attackers. If an attacker can steal the valid cookies from a victim's session, then the attacker can hijack the victim's session (Gupta & Sharma, Exploitation of Cross-Site Scripting (XSS) Vulnerability on Real World Web Applications and its Defense, 2012). This also could give the hacker the ability to login to a user's social networks such as Twitter, Facebook and Instagram, or other accounts.

The goals of this research are: to develop an understanding of the many risks of XSS attacks; to identify some companies affected by XSS attacks and the damages they suffered; to launch XSS attacks on a virtual machine to measure the damages they can cause; to categorize and compare potential defensive mechanisms; to identify solutions or proactive approaches to securing vulnerabilities exploited by XSS attacks; and to summarize best-practice solutions and recommendations based on the examined vulnerabilities.

The remainder of this paper is organized as follows. Section 2 introduces vulnerabilities and impacts related to XSS attacks. Section 3 describes exploitation and detection of XSS vulnerabilities. Section 4 documents XSS attack case studies. Section 5

explains the research methodology. Section 6 describes the research experiment in detail. Section 7 gives research results, discussion and recommendations. Section 8 concludes this paper.

2. VULNERABILITIES AND IMPACTS RELATED TO XSS ATTACKS

When attackers penetrate a victim's system, they have the ability to examine the system as well as to use other intranet applications. A few things a successful attacker might be able to do is take over an account, spread malicious worms, control web browsers remotely, exploit applications, and install a keylogger.

A webpage includes text and HTML markup that are available on the server and read by the client browser. Web sites that generate only static pages are able to have full control over how the client interprets these pages. Web sites that generate dynamic pages do not have complete control over how their outputs are interpreted by the client (Shanmugam & Ponnavaikko, 2008). XSS attacks could occur at the application-level when a server program (i.e., dynamic webpage) uses unrestricted input via an HTTP request, database, or files in its response without any validation, which allows malicious code injection (Mukesh Kumar Gupta, 2015). Exploitation of such vulnerabilities allows hackers to steal confidential information and execute other malicious actions. Examples of XSS vulnerabilities include failing to encode HTML outputs to the browser, and failing to validate inputs to web applications.

The effects of an XSS attack normally depend on the type of application, the functionality and data, as well as the affected user's privileges. The consequences of an XSS attack can be severe, including identity theft, confidential information retrieval, denial of service, changing the way the web browser operates, and even spreading worms that access the user's computer and view the user's browser history or remotely control the browser (Shanmugam & Ponnavaikko, 2008).

XSS attacks are so popular because they are fairly easy to launch and don't require a lot of technical skill. Some XSS attacks can be launched with merely basic knowledge of JavaScript and HTML. This makes it quite simple for attackers to learn how to carry out XSS attacks.

The capabilities of an attacker who launches an XSS attack can be quite broad. It is difficult for companies to track an XSS attack because there are so many ways an attacker can launch an XSS attack and exploit an XSS vulnerability. An attacker who exploits an XSS vulnerability typically is able to (Cross-site scripting):

- Impersonate or masquerade as the victim/user.
- Carry out any action that the victim/user is able to perform.
- Read any data that the victim/user is able to access.
- Capture the victim/user's login credentials.
- Perform virtual defacement of the website.

- Inject Trojan functionality into the website.

3. EXPLOITATION AND DETECTION OF XSS VULNERABILITIES

There has been a lot of prior research focused on determining ways XSS attacks are used to control and adjust how a webpage operates. Multiple platforms offer ways to test or exploit vulnerabilities of XSS attacks. A few such websites are Web Goat, Acunetix (Acunetix, 2014), Pentest Tools and Burp (Sarmah, Bhattacharyya, & Kalita, 2018). These options allow users to enter website addresses and have them checked for vulnerabilities. Since most XSS attacks involve JavaScript, all detection tools should be able to detect malicious JavaScript (Vonnegut, 2017). However, the security testing they can provided still will be limited to only systems the user owns or has permission to work with (Gupta & Sharma, Exploitation of Cross-Site Scripting (XSS) Vulnerability on Real World Web Applications and its Defense, 2012).

To minimize XSS attacks, organizations must assess their web application code and eliminate any XSS vulnerabilities. To successfully identify potential XSS attacks, organizations should adopt a few measures including (Laing, 2017):

- Evaluate any object that a browser may open, or that may launch a browser. This includes email messages, attachments, downloads, webpages, and any other document that contains HTML links.
- Perform rapid static analysis of each object, evaluating them for malicious capabilities and links, known attack signatures, structural deviations, and other anomalies.
- Perform full behavioral analysis by completely executing each object and testing it for evasion techniques and malicious actions.
- Monitor the network for side-effect activity created by malware operating on a network, such as code injection, malware communicating with command and control servers, and other anomalous activity.

There are additional ways to detect XSS attacks, but the methods listed above have been deemed the most effective. The lack of more efficient solutions is one of the reasons that XSS attacks are still so common today.

3.1 Cross-site Scripting Actors

Typically, there are three actors involved in an XSS attack: the victim, the hacker, and the website. The victim is the user of the website who requests pages from it using their browser. Hackers are the malicious users who find a vulnerability to exploit in a website to target users. The website delivers HTML pages to users who request them (Kallin & Valbuena, 2013). In some XSS attack cases, the victim doesn't have to request information from the webpage to be attacked. This makes XSS attacks more dangerous, especially XSS worms that self-propagate. When all three actors are implemented, it can result in a loss of confidential information, money, reputation, etc.

3.2 Preventing XSS attacks

A few methods are known to be effective against XSS attacks (Sarmah, Bhattacharyya, & Kalita, 2018). Five are summarized here.

- The first technique is ***escaping input***, which is the concept of ensuring the data an application has received is secure (i.e., cannot be inadvertently interpreted as code) before rendering it for further processing (Vonnegut, 2017). This technique also is used to encode special characters. As shown in Table 1, escaping changes specific characters that might otherwise be deciphered as harmful code by prefixing or replacing them with other characters. This helps control the information that goes to the webpage, which in turn reduces the chance of XSS attacks. If users are not allowed to add their own code or information to a webpage, a good rule of thumb is to escape all HTML, URLs, and JavaScripts (Vonnegut, 2017). If the webpage allows users to enter code or information (e.g., Facebook), it is best to use a similar approach that escapes all HTML input.

Table 1. Ways to escape special character attacks

Replace…	with
<	&lt
>	&gt
(	&#40
)	&#41
#	&#35

- Another technique is known as ***input validation***. This refers to the process of making sure input data is benign and contains no unexpected characters or malicious values that might otherwise attack a database, site or user. Input validation is an effective technique against XSS attacks because it prevents users from entering special characters into the fields altogether, instead of trying to intercept or deny resulting requests (Vonnegut, 2017).

- ***Sanitizing input*** prevents XSS attacks by combining other techniques such as escaping and validation. User input is analyzed and scrubbed clean of potentially harmful markup, effectively changing unacceptable user inputs to acceptable formats (Vonnegut, 2017). Sanitizing user input is especially helpful on sites that allow HTML markup.

- Another way to prevent XSS attacks is ***whitelisting values***. If a particular dynamic data item should only accept a handful of valid values, it is best practice for the rendering logic to permit only known allowed values (Protecting Your Users Against Reflected XSS). An example is for a webpage that expects

an "eye color" parameter to make sure only alphabetic letters – or a limited list of prescribed values – are accepted, instead of digits or other special characters. While whitelisting and input validation are more commonly associated with SQL injection attacks, they also can be used for preventing XSS attacks (Vonnegut, 2017).

- XSS attacks rely on the attacker being able to run malicious scripts on a user's webpage either by injecting inline <script> tags somewhere within the <html> tag of a page, or by tricking the browser into loading the JavaScript from a malicious third-party domain. So, the last method to prevent XSS attacks is implementing a ***content-security policy***. A content-security policy allows the creator of the webpage to specify where JavaScript and other potentially harmful methods can be launched and implemented (Protecting Your Users Against Reflected XSS). In this way a content-security policy can ensure that inline JavaScript isn't executed, which could prevent some XSS attacks.

4. CASE STUDY OF XSS ATTACKS

Members of the security community have researched numerous cases of XSS attacks. While there are many techniques to detect and prevent them, XSS attacks still affect companies and millions of their users. According to the report *Web Application Attack Statistics 2017 in Review*, XSS is used in 31% of all web attacks (Staff, 2018). The websites of many popular companies such as Google, eBay, Yahoo, Facebook and PayPal have been shown to have vulnerabilities that leave their users defenseless against XSS attacks. This project examined five documented XSS attacks as instructive use cases. Each case explains the organization that was targeted, the nature of the attack, and some suggested measures that could have prevent the attacks.

4.1 XSS Attack on MySpace (2005)

In October 2005, an XSS worm attacked a popular social networking website known as MySpace. Samy Kamkar, a 19-year-old hacker created the first known XSS worm to exploit MySpace's blacklist-based validation mechanism (Dabirsiaghi, 2008). What made this attack so important is that it didn't need user input – it spread on its own – and it consequently popularized XSS attacks. Within 24 hours, the attack affected over one million MySpace users. Although Kamkar's worm was harmless in theory, MySpace had to briefly shut down to fix the problem that allowed the worm to self-propagate, resulting in hours of lost production and MySpace becoming a victim of the attack right along with all the affected users.

Kamkar was able to penetrate MySpace's system by uploading an infected JavaScript to his profile, which then retrieved the user identity of the victim from the HTML source using the DOM (Richie, 2007). The attack itself was possible because an HTTP GET parameter was accepted without proper input validation checks and then echoed back to the user. If a secure Hypertext Transfer Protocol (HTTPS) had been implemented, this attack could have been prevented. (Richie, 2007). In retrospect, there

were many opportunities to prevent this attack. MySpace was capable of filtering the JavaScript, but failed to do so. Whitelisting and output escaping also could have prevented this attack. Implementing a content security policy could have blocked Kamkar from altering the code for his profile.

4.2 XSS Attack on PayPal (2006)

In June 2006, PayPal fell victim to an attack that had the potential to affect over 200 million users. Although the code from the attack was never released, it was said to be an XSS attack. The attacker inserted malicious code to retrieve confidential user information (Borg, 2006) (Seals). The attacker targeted users by sending an email stating their PayPal account had been disabled, and providing a link that allegedly would forward them to a solution. Instead, the link pointed to a malicious URL hosted on the legitimate PayPal website that asked for the user's social security number, credit card number, PIN, and other personal information (Borg, 2006).

This attack hinged on the attacker's malicious code being saved into the web application repository by the server, and then launched on the victim's browser (Kour, 2016). This was possible because PayPal's Web Application Firewall (WAF) was outdated, and they were not filtering for malicious JavaScripts. PayPal never revealed the amount of revenue it lost or the number of customers affected by this XSS attack. To prevent similar attacks in the future, PayPal could invest in an Acunetix Web Vulnerability Scanner which checks websites for exploitable vulnerabilities.

4.3 XSS Attack on Orkut (2010)

In 2010, an XSS vulnerability was exploited on Google's social media platform Orkut. It was a fast-moving malicious JavaScript that forced users to post specific content. This attack affected a victim's profile, then spread through all their friends, who spread it to all their friends, and so on, ultimately affecting over five million users all over the world. It spread overnight and infected users who viewed emails or Orkut messages carrying the malicious payload (Higgins, 2007). The email addresses of all victims were made available to the attacker which left them vulnerable to further attacks. The vulnerability was fixed within a few hours and the affected profiles were repaired. Since the accounts were connected to Google, all users were instructed to reset their passwords.

Persistent XSS vulnerabilities like the one exploited in this attack are the result of failing to properly sanitize input into forms. This allows attackers to insert malicious code into pages (Constantin, 2010). Validating input could have been used to prevent this XSS attack by making sure only legitimate data was being input into the webpage forms. Another effective measure would have been a content security policy that could have prevented the malicious JavaScript from being loaded and executed in the first place. In addition, Orkut didn't use a secure protocol; if they had, the breach also may have been prevented.

4.4 XSS Attack on Amazon (2013)

In December 2013, Amazon became a victim of a persistent XSS attack that left their customers vulnerable to their information being stolen. The vulnerability affected Kindle e-book readers. The malicious code was injected through e-book metadata. For example, the attacker could add a book title containing code such as *"<script src="https://www.example.org/script.js"></script>"* (Kovacs, 2014). This allowed cookies to be accessed by the attacker, which could lead to personal information being compromised, such as usernames and passwords. This vulnerability affected everyone who used the Kindle library to keep their e-books.

Amazon took a little over a month to respond to this vulnerability. The attack damaged Amazon's reputation, and likely affected their revenue: users were afraid to download the Kindle application due to all the bad press, which meant they weren't buying eBooks for the reader either. This vulnerability could have been prevented by using intrusion detection systems, which wouldn't have allowed the attacker to insert malicious data via e-book metadata. Likewise, validating input could have prevented the malicious code injection.

4.5 XSS Attack on Twitter (2014)

In 2014, an XSS vulnerability was found in TweetDeck, an application within Twitter (Cross-Site Scripting (XSS) Found in Tweetdeck, 2014). The attacker simply tweeted malicious JavaScript to make users automatically retweet tweets, and it began to regenerate. At the time, Twitter had over 50 million users, and over 15 percent of them were affected. Users were concerned that their accounts had been hijacked. The vulnerability remained on the site for so long that some users began to use it to implement harmful JavaScript and possibly steal other users' credentials. It is very likely that many users' information was stolen, although this was never confirmed.

This attack was possible because Twitter didn't have an updated WAF to filter code before it is processed to the webpage. To prevent this attack, Twitter could have practiced sanitizing input, which would have prevented the attacker from implementing the code in the browser. Twitter also could have protected their restricted servers by implementing separation of duty and access so that third parties would not be able to access them.

5. RESEARCH METHODOLOGY

This research explores the relationship between web application vulnerabilities and XSS attacks. The first four sections of this paper introduced details about the elements of typical XSS attacks – including the actors – as a basis for understanding the motivation and importance of this research. The experimental component of this study includes launching XSS attacks modeled after known attacks in a virtual environment to gain insight about ways they could have been prevented, and finally proposing a list of alternative solutions to prevent them. The attacks included were reflected XSS attack, persistent XSS attack, stealing cookies, and keylogging. Each of these attacks is related in some way to the attacks explored in the XSS use cases examined earlier.

The following methodology is followed in conducting the research and subsequent experimentation:

- Study and analyze previous XSS attack cases.
- Build fundamental virtual systems including web servers, SQL and several other related servers if needed, and some web users to mimic real-world systems.
- Introduce/inject well-known XSS attacks to the fundamental systems.
- Measure damages caused by the XSS attacks.
- Suggest solutions to prevent such XSS attacks.

6. CONDUCTING THE EXPERIMENT

6.1 Studying and Analyzing Previous XSS Attack Cases

There have been a few cases in which an XSS has affected thousands of people by exploiting vulnerabilities. The knowledge and experience gained from these cases can help implement XSS attacks in the virtual environment. Every XXS attack explored in this research exploits a vulnerability within a website. Most of the discussed XSS attacks could have been prevented if HTTPS were used instead of HTTP. HTTP doesn't encrypt data, leaves users open to attacks, and can present altered data to end users. Systems that use HTTP transmit data on port 80 and are vulnerable to information being intercepted.

HTTPS provides an authenticated server along with protection from hackers and data encryption. HTTPS transmits data on port 443 and uses a Secure Sockets Layer (SSL), which establishes encryption between the server and web browser. Transport Layer Security (TLS) is a cryptographic protocol that provides end-to-end communications security over networks and is widely used for internet communications and online transactions (Kerravala, 2018). The PayPal and Twitter attacks described earlier might have been prevented if the TLS protocol had been implemented (Kerravala, 2018).

The attacks discussed in this research are exploited by an attacker targeting the server and bypassing validation mechanisms. Each company used a server to process JavaScript after performing input filtering and other XSS prevention techniques; however, the attacks still occurred. The use of separation of duties in these attacks would have ensured that the attacks were detected quickly and protection mechanisms were enacted to protect the server. Separation of access would have ensured that no third parties could access the server, thereby keeping user information secure.

Table 2 shows the targets of XSS attacks from the previous section, the types of attacks that occurred, and security protocols that might have prevented them. None of the attacks listed in Table 2 were detected immediately. If these companies had used appropriate security protocols/tools, these attacks might have been prevented, or perhaps less effective.

Table 2. XSS attacks and protocol to possibly prevent them

Targeted Website	Type of XSS attack	Brief Description	Vulnerabilities	Protocols/tools to possibly prevent attack
MySpace (2005)	Stored XSS Worm	Worm exploited a flaw in MySpace's filter, which allowed hacker to inject code into a user's profile.	Located within OS of the server, HTTP, and web application	HTTPS, WAF
PayPal (2006)	Stored XSS attack	Vulnerability located in PayPal allowed an attacker to steal confidential information from users.	Outdated Web Application Server	HTTPS, WAF
Orkut (2010)	XSS worm	Self-propagating worm affected users by spreading malicious code to each profile who viewed the affected profile.	Vulnerable Web Server, Use of HTTP	HTTPS, WAF
Amazon (2013)	Stored XSS attack	Vulnerability allowed hackers to steal cookies, and user credentials.	Vulnerable Web Server, Outdated WAF	HTTPS, WAF
Twitter (2014)	Stored XSS attack	An XSS attack on Twitter caused users to post things without their permission.	Within the web server, Outdated Web server, No SSL Certificate	HTTPS, WAF

6.2 Building XAMPP on Virtual Machine for Simulating XSS Attacks

The following testbed was built for the purposes of this experiment: a virtual machine hosted in VirtualBox using XAMPP (XAMPP Apache + MariaDB + PHP + Perl, n.d.) under the domain "http://localhost". VirtualBox is software that allows users to run multiple operating systems in a simulated environment. This is beneficial when deploying XSS attacks so that the host operating system will not be damaged. VirtualBox allows users to select the storage and memory size needed for a virtual machine.

To implement a VirtualBox system loaded with Windows 10 to test the XSS attacks, download VirtualBox for Windows from the VirtualBox website (Mac, Linux, and Solaris downloads also are available). Next download the Windows 10 ISO file directly from the Microsoft website, and install it to emulate the Windows environment. Once Windows is successfully installed onto the virtual machine, download XAMPP for Windows from the Apache Friends website.

When XAMPP is installed, the user can select which components to install to test XSS attacks. Only Apache, PHP and MYSQL were needed for this project. The default ports remain the same for launching attacks, and the XAMPP control panel is right-clicked

to run the program as an administrator. Webpages can be added to the XAMPP folder to add files to the local host. For this project HTML, PHP and JavaScript files were used to implement the attacks.

6.3 Create Simulated XSS Attacks on an XAMPP System

In this research, several well-known XSS attacks were introduced into a web server. To implement these attacks, a vulnerable webpage is created and launched on the local host. Knowledge of JavaScript and MySQL are needed to implement these attacks. Four XSS attacks are launched on the XAMPP system to mimic damaging XSS attacks:

- **Reflected XXS Attack**: Reflected XSS is the most common XSS attack method. When a reflected XSS attack occurs, malicious code is reflected off the web server. The attacker injects code into the web server and the victim's browser executes the code. Common reflected XSS tactics include stealing cookies, redirecting to a phishing site, and making the user complete a task. For this experiment, code is injected into a webpage to show that the webpage is vulnerable. When the following code is injected into the text box

  "<script>alert("XSS") </script>".

  an alert text box pops up showing that the webpage is vulnerable. After determining that the webpage is vulnerable, hackers are able to launch almost any XSS attacks into the search bar. Figure 1 shows the URL string when a malicious script was injected into a search box implemented on the server.

```
localhost/xss/2/index.php?search=%3Csecipt%3Ealert%28%22XSS%22%29%3C%2Fscript%3E
```

Figure 1. URL of a malicious string inserted into a search box

- **Persistent XSS Attack**: In a persistent XSS attack, the malicious code comes from the database. These attacks often occur on blogs, forums, and web browsers. The code forces the webpage to redirect the user to another website. Following this command, the JavaScript will return the user to the webpage containing the script. Attackers use this technique to redirect users to fake websites to ask for user information such as credit card, social security numbers, and other confidential information. Figure 2 shows a JavaScript being stored on the webpage. If users visit the webpage after the implementation of this code, they will be directed to a Google search of Norfolk State University.

```
<script>
window.location='https://google.com/search?q=norfolk+state+university'
</script>
```

Figure 2. A malicious JavaScript inserted into the webpage

- **Stealing Cookies XSS Attack**: A cookie is a tiny piece of information that is sent from a website and stored on the user's computer by the web browser. Websites use cookies to remember certain details about a user, and in other situations such as adding items to a shopping cart. Attackers can use this data maliciously to steal sensitive information like credit card numbers, browsing history and email information. Figure 3 shows a JavaScript to capture the cookies of a user who views the webpage. When this code is implemented, the attacker can view confidential information about a user and perform any actions for which the user has permissions (Fake WordPrssAPI Stealing Cookies and Hijacking Sessions, 2017).

```
<script>
window.location='http://localhost/cookiemonster.php? cookie=' +eacape(document.cookie.)
</script>
```

Figure 3. A malicious JavaScript to capture the cookies from a user

- **Keylogging XSS Attack**: Keylogging often is used in XSS attacks to capture the user's keystrokes to steal usernames, passwords, social security numbers, addresses, etc. Keylogging attacks are so successful because they are difficult to detect. JavaScript, PHP, and HTML code can be used to implement this attack. Figure 4 shows a JavaScript used to launch a keylogging attack on the user.

```
var keys=''
document.onkeypress = function (e) {
   get = window.event?event: e;
   key = get.keyCode?get.keyCode:get.charCode;
   key = Sring.formCharCode(key);
   keys+=key;
}
window.setInterval(function() {
   if(keys != '') {
      new Image().src =  'http://27.0.0.1/xss/7/exploit/exploit.php?keylog='+keys;
      keys = ''
   }
}, 1000);
```

Figure 4. A malicious JavaScript used for keylogging

6.4 Measuring Damages of XSS Attacks

In this research, the impact of each XSS attack was measured quantitatively and qualitatively. For the quantitative measure, NIST CVSS 3.0 ratings are used. The qualitative measure determined whether the XSS attack violates the confidentiality, integrity, availability (CIA) triad. *Confidentiality* refers to limiting information access and disclosure to only authorized users, as well as preventing access by, or disclosure to, unauthorized users. *Integrity* refers to the consistency and authenticity of information. *Availability* refers to the ability for authorized users to access information resources when they need them.

Figure 5 details the base score equation, which determines the scores for each attack by using the CVSS Calculator by the National Vulnerability Database. The formulas for the base score and for the exploitability and impact sub-scores, are based on expert opinions rather than formal derivations (Rouse, 2016) (Common Vulnerability Scoring System v3.0: Specification Document) (Younis & Malaiya, 2015).

The numbers are generated from the exploitability and impact group measures which include Attack Vector, Attack Complexity, Authentication, Confidentiality, Integrity, and Availability. *Attack Vector* refers to the vulnerability being exploited on the network, adjacent network, local, or physical network. *Attack complexity* is split into two categories, low and high, which are based on the difficulty of the attack. *Privileges Required* refers to what access the hacker has at the time of attack. *User Interaction* refers to whether the vulnerability can be exploited without the user communicating. *Scope* is "unchanged" when the impacted component and vulnerable component are the same; whereas scope is "changed" when the impacted component and vulnerable component are different. These six metrics were represented by fixed numerical values to determine the base score (BS) using the base equation (Rouse, 2016) (Common Vulnerability Scoring System v3.0: Specification Document) (Younis & Malaiya, 2015).

The BS is a function of the *Impact* and *Exploitability* sub-score equations. The BS varies on each attack, based on the impacts it causes. Formally, *Scope* refers to the collection of privileges defined by a computing authority (e.g., an application, an operating system, or a sandbox environment) when granting access to computing resources (e.g., files, CPU, memory, etc.). The two CVSS sub-scores range between 0.0 and 10.0. A CVSS score from 0.0 to 3.9 corresponds to Low severity, from 4.0 to 6.9 corresponds to Medium severity, and from 7.0 to 10.0 denotes High severity (Rouse, 2016) (Common Vulnerability Scoring System v3.0: Specification Document) (Younis & Malaiya, 2015).

The Base Score (BS) is a function of the Impact and Exploitability sub score equations. It is defined as:

If (Impact Sub Score <= 0) {
$Base\ Score = 0$
else
Scope Unchanged: $Base\ Score = Roundup(Minimum[(Impact + Exploitability), 10])$
Scope Changed: $Base\ Score = Roundup(Minimum[1.08 \times (Impact + Exploitability), 10])$
}

and the Impact Sub Score (ISC) is defined as,

Scope Unchanged: $ISC = 6.42 \times ISC_{Base}$
Scope Changed: $ISC = 7.52 \times [ISC_{Base} - 0.029] - 3.25 \times [ISC_{Base} - 0.02]^{15}$

Where,

$ISC_{Base} = 1 - [(1 - Impact_{Conf}) \times (1 - Impact_{Integ}) \times (1 - Impact_{Avail})]$

And the Exploitability Sub Score is,

$8.22 \times AttackVector \times AttackComplexity \times PrivilegeRequired \times UserInteraction$

Figure 5. Equation used to calculate the Base Score

The four XSS attacks were analyzed using the BS Formula based on exploitability and impact metrics in which the attacks were completed.

- **Reflected XSS Attack**: Figure 6 shows the base scores for the reflected XSS attack. The vulnerability is exploitable with network access. The attack complexity is low, and low privileges are required. User interaction is required and the scope is changed. The confidentiality and integrity impacts are low, while availability is not impacted.

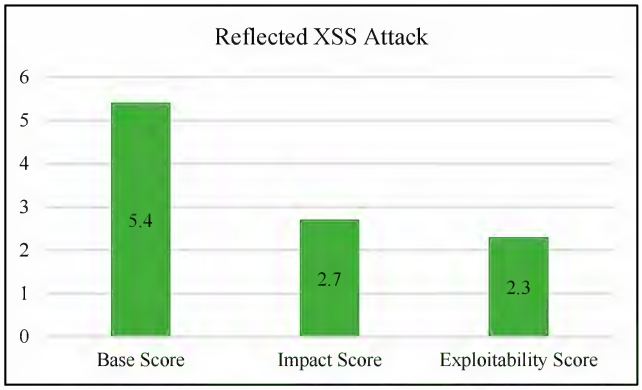

Figure 6. Base scores for a reflected XSS attack

- **Persistent XSS Attack**: Figure 7 shows the base scores for the persistent XSS attack. The vulnerability is exploitable with network access. The attack complexity is low, and no privileges are required. User interaction is required and the scope is changed. The confidentiality and integrity impacts are low, while availability is not impacted.

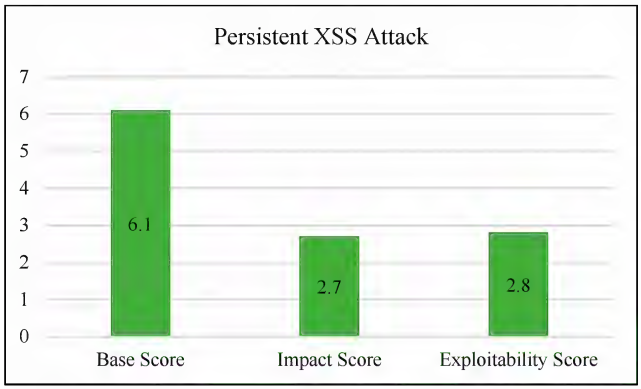

Figure 7. Base scores for a persistent XSS attack

- **Cookies Stealing XSS Attack**: Figure 8 shows the base scores for a cookie stealing XSS attack. The vulnerability is exploitable with network access. The attack complexity is low, and no privileges are required. User interaction is required and the scope is changed. The confidentiality and integrity impacts are low, while availability is not impacted.

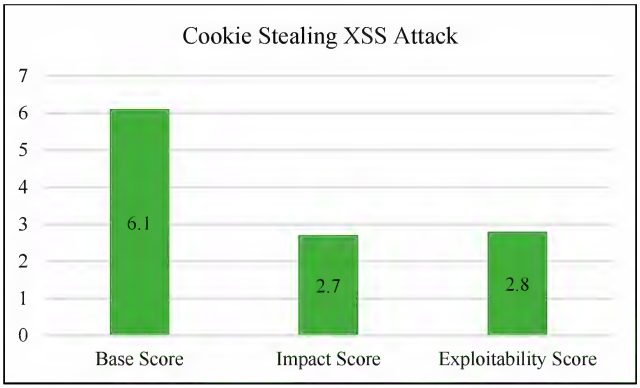

Figure 8. Base scores for a cookie stealing XSS attack

- **Keylogging XSS Attack**: Figure 9 shows the base scores for a keylogging XSS attack using the Base Score Formula. The vulnerability is exploitable with local access. The attack complexity is low, and no privileges are required. User interaction is not required and the scope is changed. The confidentiality impact is high and integrity impacts is low, while availability is not impacted.

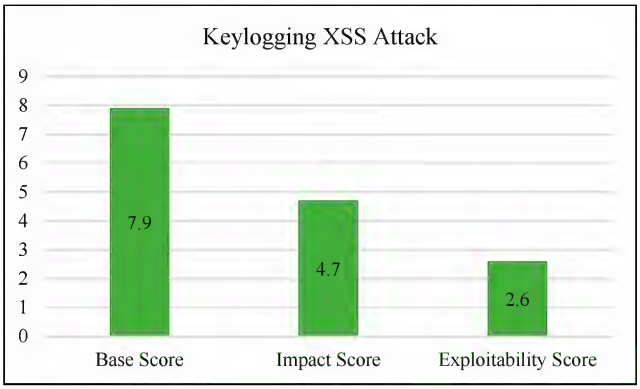

Figure 9. Base scores for a keylogging XSS attack

7. RESEARCH RESULTS, DISCUSSIONS AND RECOMMENDATIONS

7.1 Results

This section summarizes the results of the study. Known XSS attacks were scrutinized to determine the impacts they cause and how the vulnerabilities are exploited. Because many websites still have vulnerabilities, a virtual machine hosted in VirtualBox using XAMPP was deployed to test XSS attacks. Once these attacks are launched on the

virtual machine, their impacts can be measured using the CVSS Calculator. This tool details how to score CVSS vulnerabilities and interpret their base scores. This research was completed to test vulnerabilities in webpages and to propose prevention techniques.

Section 6.4 shows the impact results of each XSS attack quantitatively and qualitatively. These numbers were generated using the BS formula (see Figure 5). The numbers are formulated from exploitability and impact metrics that are determined by the National Vulnerability Database. The BS formula determines that a reflected XSS attack has the least impact, while keylogging has the greatest effect. Table 3 rolls up the impact findings for the four analyzed attacks.

Table 3. Summary of XSS Attack Damages

XSS attack	Base Score	Impact Score	Exploitability Score	Confidentiality Impact	Integrity Impact	Availability Impact
Reflected	5.4	2.7	2.3	Low	Low	None
Persistent	6.1	2.7	2.8	Low	Low	None
Cookie Stealing	6.1	2.7	2.8	Low	Low	None
Keylogging	7.9	4.7	2.6	High	Low	None

All attacks required user interaction and the scope was changed
0.0 to 3.9 = low; 4.0 to 6.9 = medium; 7.0 to 10.0 = high

7.2 Discussion

- ***Reflected XSS Attack***: This is a simple attack method used to determine if a website is vulnerable. This attack has the lowest base score which is to be expected since it only tests the vulnerability of a webpage.

- ***Persistent XSS Attack***: This attack is more advanced than a reflected XSS attack. The hacker inserts code into the website and it redirects users to that website. This attack has a medium base score.

- ***Cookies Stealing XSS Attack***: These attacks can be used to get a user's cookies, which can be used to view browsing history, usernames, passwords, etc. Although it can be very malicious, this attack has a medium base score.

- ***Keylogging XSS Attack***: This attack is commonly used when trying to duplicate the keystrokes of users without their knowledge. It has the highest base score which means it is the most impactful attack. This technique indeed is very successful and difficult to detect, making it the most aggressive of the four XSS attacks analyzed.

7.3 Recommendations

The findings in this study suggest the following recommendations to help avoid XSS attacks:

- ***Validate, escape and sanitize user input.*** These methods make sure input data, as well as HTML, URLs and JavaScript, is benign and contains no unexpected characters or malicious values that might otherwise comprise an XSS attack. This approach is especially recommended for avoiding XSS attacks in forms and text boxes which can be used to launch stored XSS attacks.

- ***Use web vulnerability checking tools.*** Various websites and technologies help check for website vulnerabilities; a few were mentioned in Section 3. Since most XSS attacks involve JavaScript, all detection tools should be able to detect malicious JavaScript.

- ***Use an up-to-date WAF***. This will filter code before it is processed to the webpage.

- ***Use relevant security protocols.*** The analysis of historical XSS attacks presented in Section 6 mentioned a number of security protocols that might have prevent attacks. Using HTTPS instead of HTTP provides an authenticated server along with protection from hackers and data encryption.

- ***Implement content security policy, separation of duties/access.*** The use of appropriate policies and access controls related to security helps limit where JavaScript and other potentially harmful methods can be launched and implemented, and which assets individuals or third parties can access and use. Appropriate policies not only help prevent XSS attacks, but also enhance the security of vital servers and user information. If breaches are successful, effective policies help ensure attacks are detected and repaired quickly.

8. CONCLUSIONS

XSS attacks are very common and threatening web application attacks that can expose a user or a company's resources and leave them open to further attacks. XSS attacks are experienced in various forms such as pop-up windows, viruses, worms and account hijackings. Although a fair amount of research has been attempted to mitigate XSS attacks, there still is a lack of systematic study and investigation related to this issue.

To achieve project goals, XAMPP was built on a virtual environment to study and investigate several well-known XSS attacks. Attack details were studied and their impacts were measured. This research also addresses solutions and recommendations for mitigating XSS attacks. Characterizing vulnerabilities and attacks using standard means like the CVSS calculator can help to rank order and prioritize defensive measures when resources

are constrained. Future works can conduct similar studies on additional XSS variants like DOM-based XSS and other known attack families.

In conclusion, this project helps to fill the XSS prevention gap through the following research steps:

- Studying and analyzing several well-known XSS attack cases.
- Building fundamental virtual systems to mimic real world systems.
- Injecting XSS attacks into the fundamental systems.
- Measuring damages caused by the XSS attacks, and
- Providing solutions to prevent such XSS attacks in the future.

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The Diversity and Distribution of Spiders (Arachnida: Araneae) Along an Outdoor – Indoor Habitat Gradient: Preliminary Findings from Piedmont Virginia

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ABSTRACT

Although the United States supports a considerable diversity of spiders, some aspects of spider habitat use and niche specialization are poorly documented. Specifically, little attention has been given to explore how urban development affects the diversity and abundance of arthropods. We sampled spiders along an outdoor – indoor habitat gradient at Longwood University to understand the impact of urbanization on species diversity and abundance. We found 50 taxa of spiders belonging to 43 genera and 16 families. Overall, the most abundant spider family across three sampling sites was *Araneidae* (orb-weavers; 18.2%) followed by *Lycosidae* (wolf spiders; 14.8%), *Salticidae* (jumping spiders; 13.6%) and *Linyphiidae* (sheetweb spiders; 12.5%). We found the highest species richness, spider abundance, and Shannon-Wiener diversity from Lancer Park (i.e. outdoors habitat), followed by the habitats associated with outside of the science center building (i.e. marginal habitat) and the lowest spider diversity inside the science building (i.e. indoors habitat). We also found a strong positive correlation between overall spider diversity and air temperature for outdoors and marginal habitats, but no correlation with relative humidity. Our study adds original knowledge about habitat use of spiders along an outdoor - indoor habitat gradient and arthropod use of indoor biome. More importantly, our study stresses the need for more extensive systematic studies to fully understand how spatial and temporal variation of arthropod diversity and abundance may be influenced by alterations of habitats by humans through urbanization.

INTRODUCTION

Spiders are one of a few cosmopolitan groups of organisms utilizing a range of habitats from hot deserts to the cold Arctic (Foelix 2011) to urban habitats with man-made structures. Worldwide, there are about 48,000+ (World Spider Catalog 2019) formally described species of spiders including at least 3,800 species in North America (Bradley 2013). Although spiders are ubiquitous, little attention is typically given by the ecology research community, to study their diversity, biology, and ecology, possibly due to their small size, seemingly secretive behavior, lack of information on true diversity and

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subjective fear of spiders in general. With that being said, the true diversity of spiders in the eastern United States may be poorly documented (Howell and Jenkins 2004).

Spiders play important ecological roles in their habitats, mainly as predators and prey. They are important predators in the natural ecosystem (Foelix 2011, Mallis and Rieske 2011) and are typically generalist predators, many feeding on different terrestrial arthropods, but some are specialists (Mallis and Hurd 2005, Mallis and Rieske 2011). Agriculturally, spiders are very helpful in limiting the amount of pest populations in crops. It is estimated that spiders consume 400-800 million tons of prey annually (Mallis and Hurd 2005). Spiders are very efficient as natural pest control agents, hence some rice farmers in Asia do not use pesticides (Nyffeler and Benz 1987). Additionally, spiders have complex trophic networks and may belong to more than one trophic level based on their diet and size (Wise et al. 1999). For example, larger wolf spider species tend to prey on herbivores while smaller wolf spiders in leaf litter prey on detritivores, fungivores, and herbivores (Mallis and Hurd 2005). On the other hand, spiders are also a source of food for many larger organisms including birds (Rogers et al. 2012).

Although the United States supports a considerable diversity of spiders, some aspects of spider habitat use and niche specialization are poorly documented (Howell and Jenkins 2004). A recent study concluded that eastern hemlock canopies were more diverse than deciduous canopies for spiders (Mallis and Rieske 2011). Mallis and Hurd (2005) reported 50 species of ground-dwelling spiders from a successional gradient of habitats in southwestern Virginia including habitat specialists and generalists. Smith et al. (2018) compared spider diversity between mesic and xeric habitats in Pike County, Alabama, and reported 82 species belonging to 24 families (Smith et al. 2018). Some relatively unexplored aspects of spider ecology are the use of man-made structures as habitat by spiders and how urbanization affects them.

With the rapid expansion of human population, the impacts of urbanization generally cause loss of native species diversity (Blair 1996, Gagne and Fahrig 2011). However, urbanization may also promote a few urban-adapted taxa and lead to biotic homogenization (Blair 1996). Overall, little attention has been given to explore how urban development affects the diversity and abundance of arthropods (McIntyre 2000, Shochat et al. 2004) despite the ubiquitous nature of arthropods in human dwellings. Spiders are one of many arthropod groups commonly associated with urban habitats and human dwellings. Shochat et al. (2004) showed that the transformation of a xeric natural habitat into an urban habitat caused reduced spider diversity and the establishment of a few spider taxa that can tolerate the new urban setting. Additionally, a recent study that analyzed the diversity of the indoor arthropod biome found that spiders represent nearly one-fifth of the indoor arthropod diversity (Bertone et al. 2016). Therefore, the differences in diversity and abundance of spiders may reflect the changes in trophic structure in human-altered systems (Shochat et al. 2004).

In this study, we conducted a survey of spiders along an outdoor – indoor habitat gradient at Longwood University to understand the impact of urbanization on species diversity and abundance. Specifically, our goals included 1) comparing and contrasting

spider diversity from three distinct habitats covering an indoor-outdoor habitat gradient, 2) exploring the relationships between environmental conditions and the diversity of spiders, and 3) generating a preliminary species list for Longwood University premises. We predicted that indoor habitats would support less diversity of spiders compared to outdoor and marginal habitats. Additionally, we expected a positive correlation between spider diversity and two environmental variables, temperature and relative humidity.

MATERIALS AND METHODS

Study Area

This study was conducted at Longwood University in Farmville, Virginia (37.2972971,-78.3972648). We selected three specific habitats to represent an outdoor – indoor habitat gradient. We selected the lowland floodplain of the Buffalo Creek at Lancer Park as the outdoor habitat. This relatively small land area (0.12 km^2) represents a diverse array of both aquatic and terrestrial habitats including a third order stream, a series of seasonal pools, several man-made ponds, eastern deciduous forests, grasslands and hedge habitat, and some buffer habitat with parking lots and roads. We specifically sampled grassy areas with shrub or tree margins at Lancer Park (i.e. outdoor habitat). Additionally, we sampled inside the Chichester Science Building as the indoors habitat including classrooms, lab spaces, and stairwells (i.e. indoor habitat), while habitats outside Chichester Science Building including walls, windows, and adjacent vegetation up to 5 m from the building (i.e. marginal habitat) (Fig. 1). We sampled all study areas in March and April of 2018. Outdoor and marginal habitats were sampled four times, but indoor habitats were sampled only twice due to logistical limitations.

Figure 1. The major sampling locations for this study. The Lancer Park flood plain (A) served as the outdoor habitat and the Chichester science building (B) served as the indoor and marginal habitats.

Field Data Collection and Spider Identification

We collected spiders by opportunistic sampling (Motley et al. 2017) within each sampling location using an array of sampling methods during day time. Visual observations and hand picking were mainly employed in indoor habitats and additionally, sweep nets and beat sheets were used for outdoor sampling. These methods allowed us to collect spiders from diverse microhabitats. Sampling was conducted for two hours at Lancer Park and another two hours covering inside and outside of the Chichester Science building. All spiders were photographed and released back to the original capture locations. Environmental data such as temperature and humidity were collected at capture locations using the RockyMars ® RT36 temperature and humidity meter. Spiders were identified to

the lowest possible taxa (i.e. genus or species) using field guides and identification keys provided by Howell and Jenkins (2004), Gaddy (2009), Bradley (2013) and Ubick et. al. (2017).

Data Analysis

Overall relative abundance of spiders for each family was estimated by dividing the pooled number of individual spiders belonging to a given family by the total number of spiders. Shannon-Wiener diversity index (H') and Simpson's dominance index (D) were computed for each sample using the following formulae (Krebs 1999) to estimate overall diversity and dominance of spider communities in each sample respectively.

$H' = -\sum (P_i * \ln P_i)$

$D = \sum (P_i)^2$

Where, Pi = fraction of the entire population made up of species i.

The mean differences between location and sampling dates for 1) number of species, 2) overall abundance, and 3) Shannon-Wiener diversity index were analyzed using non-parametric Kruskal-Wallis rank sum test. We used this test due to the small sample size of our data. The effects of measured environmental conditions on overall spider diversity calculated by the Shannon-Wiener diversity index were analyzed using simple linear models considering temperature and relative humidity as predictor variables. All statistical analyses were conducted using R statistical software program (R Core Team 2016).

RESULTS

Overall Spider Diversity

We found 88 individual spiders belonging to 50 taxa under 43 genera and 16 families (Table 1, Appendix 1). The highest diversity of spiders was reported in Lancer Park (i.e. outdoors habitat) and the least diversity inside the science building (i.e. indoors habitat). The habitats associated with the outside of the science building (i.e. marginal habitat) had an intermediate level of spider diversity. Out of 36 total spider taxa reported from Lancer Park, spiders belonging to 8 families, 27 genera and 33 taxa were restricted only to Lancer Park. Only three spider taxa were reported from inside the science building and 2 taxa belonging to a single genera (*Pholcus*) and a single family (Pholcidae; cellar spiders) were restricted to only inside of the science building. Additionally, out of 14 total spider taxa reported, 11 taxa belonging to 9 genera were restricted only to marginal habitats associated with outside of the science building (Fig. 3).

Table 1. The checklist of spiders reported during this study from both outdoor and indoor habitats. The species reported only from indoors are marked with an asterisk.

Family	**Common name**	**Number of Genera**	**Number of Species**
Anyphaenidae	Anyphaenid sac spiders	1	1
Araneidae	Orb-weaver spiders	9	9
Clubionidae	Sac spiders	1	1
Gnaphosidae	Ground spiders	1	1
Linyphiidae	Sheetweb spiders	3	3
Lycosidae	Wolf spiders	6	7
Oxyopidae	Lynx spiders	1	2
Philodromidae	Running crab spiders	1	2
Pholcidae*	Cellar spiders*	1*	2*
Pisauridae	Nursery web spiders	2	3
Salticidae	Jumping spiders	8	9
Tetragnathidae	Long-jawed orb-weavers	1	1
Theridiidae	Cobweb spiders	4	4
Thomisidae	Crab spiders	2	3
Uloboridae	Hackled orb-weavers	1	1

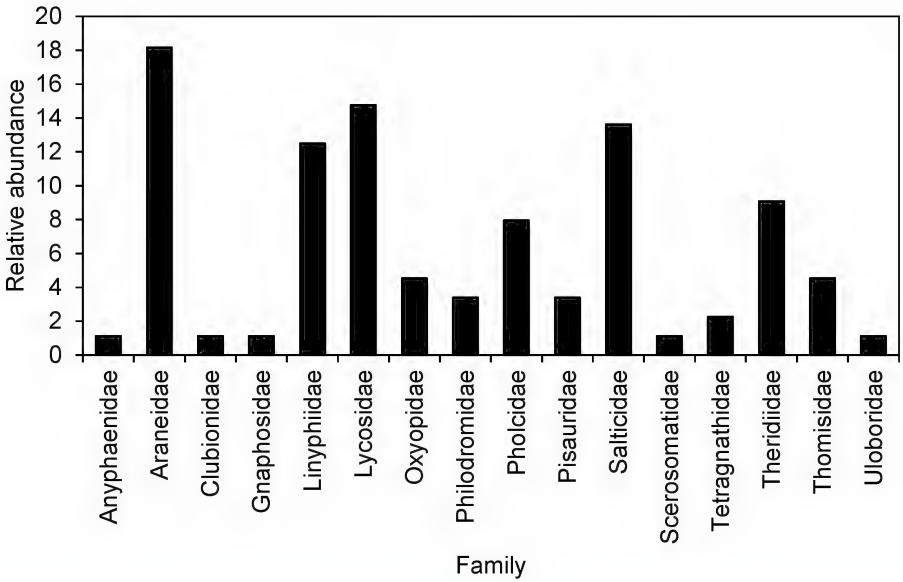

Figure 2. The relative abundance of spider families reported across Lancer Park (outdoor habitat), inside of the science building (indoor habitat) and outside of the building (marginal habitat).

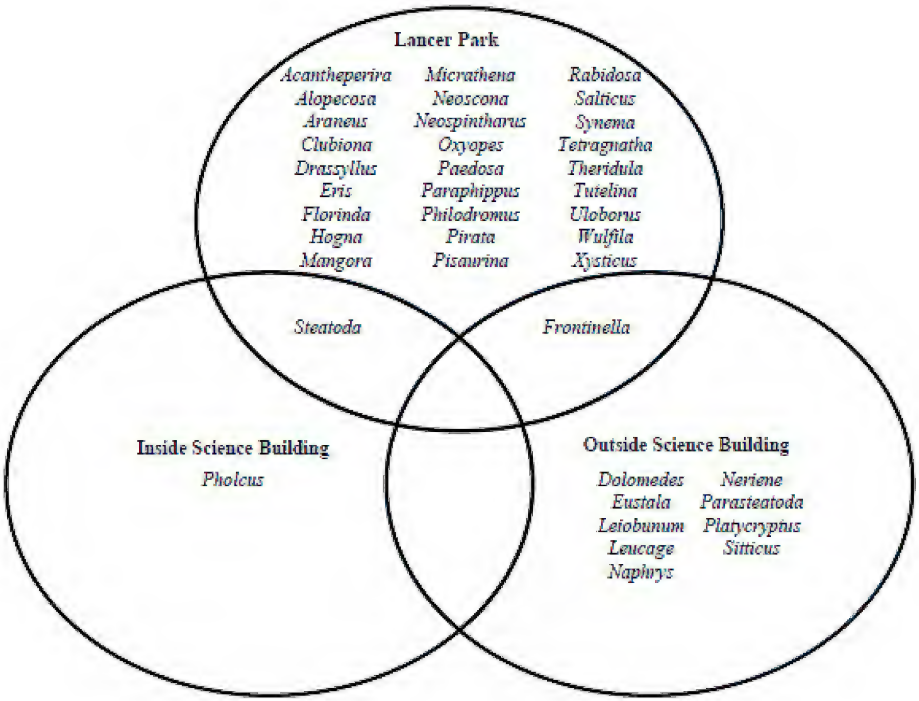

Figure 3. Venn diagram summarizing unique genera and overlapping genera of spiders from Lancer Park (outdoor habitat), inside of the science building (indoor habitat) and outside of the building (marginal habitat).

Overall, the most abundant spider family across three sampling sites was Araneidae (orb-weavers; 18.2%) followed by Lycosidae (wolf spiders; 14.8%), Salticidae (jumping spiders; 13.6%) and Linyphiidae (sheetweb spiders; 12.5%) (Fig. 2). The least abundant families were represented by single specimens for Anyphaenidae (Anyphaenid sac spiders), Clubionidae (sac spiders), Gnaphosidae (ground spiders), and Uloboridae (hackled orb-weavers) (Fig. 2). Typically, we found single individuals of spiders belonging to each species from each sampling location during each sampling session, indicating low abundance. However, relatively higher abundances (i.e. more than 3 individuals per sampling session) were reported for families Linyphiidae (sheetweb spiders), Lycosidae (wolf spiders), and Oxyopidae (lynx spiders) for Lancer Park; Pholcidae (cellar spiders) for inside science building; and Araneidae (orb-weavers), Linyphiidae (sheetweb spiders), Salticidae (jumping spiders) and Theridiidae (cobweb spiders) for the marginal habitat. Genus *Frontinella* (Bowl and Doily spider, *F. pyramitela*) was shared between the Lancer Park and outside of the science building, while genus *Steatoda* (False black widow spider, *S. grossa*) was shared between the Lancer Park and inside the science building (Fig. 3). None of the spider genera were shared between inside and outside of the science building

or among all three habitats. Five spider families were shared between the Lancer Park and outside of the science building (Araneidae, Linyphiidae, Lycosidae, Pisauridae and Salticidae) while none were shared exclusively between the Lancer Park and inside the science building or exclusively between inside and outside of the science building (Fig. 3). Only one family (Theridiidae; cobweb spiders) was shared among all three habitats. The very limited shared taxa among three habitats suggests unique spider assemblages in each sampled habitat (Fig. 3).

Effects of Sampling Location on Spider Diversity

Overall, we found the highest species richness, spider abundance, and Shannon-Wiener diversity from Lancer Park (i.e. outdoors habitat), followed by the habitats associated with outside of the science center building (i.e. marginal habitat), and the lowest spider diversity inside the science building (i.e. indoor habitat) (Fig. 4). On average, the highest number of species was recorded from the Lancer Park (9.75 ± 4.71), followed by the science center building outside (4.50 ± 2.18) and inside (2.5 ± 0.50). However, we did not see a significant difference of number of species among locations (chi-squared = 2.5555, d.f.= 2, p = 0.2787; Fig. 4-A), possibly due to our small sample size. A similar trend was observed for overall abundance of spiders, where the highest abundance was recorded from the Lancer Park (12.00 ± 5.07), followed by the science center building outside (8.00 ± 5.67) and inside (4.00 ± 0.00). Again, we did not see a significant difference of abundance among locations (chi-squared = 2.0124, d.f. = 2, p-value = 0.3656; Fig. 4-B). Additionally, a similar, non-significant (chi-squared = 3.1058, d.f. = 2, p-value = 0.2116; Fig. 4-C) trend was observed for Shannon-Wiener diversity with the highest diversity occurring at the Lancer Park (1.84 ± 0.51), followed by the science center building outside (1.16 ± 0.34) and inside (0.80 ± 0.24).

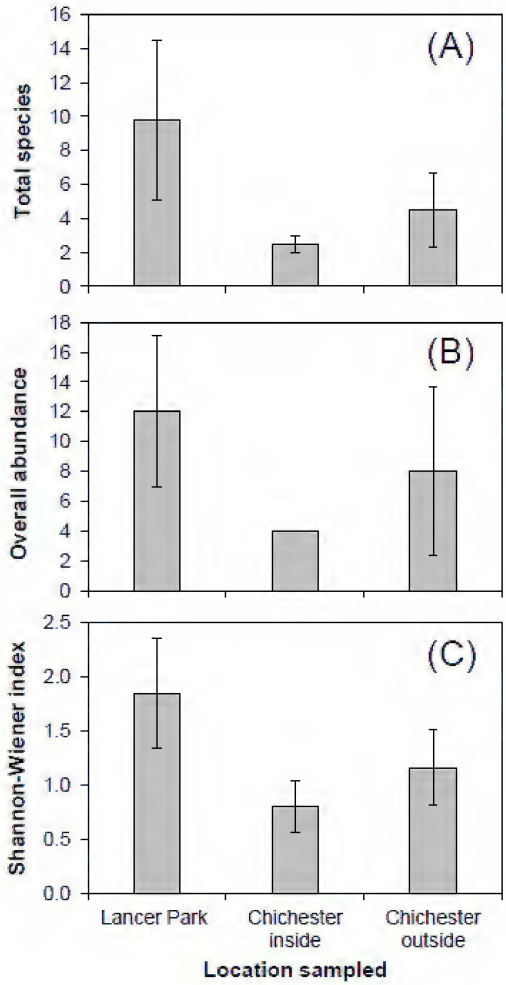

Figure 4. The average (± S.E.) number of species (A), overall abundance (B), and Shannon-Wiener diversity (C) reported for three sampling locations, Lancer Park (N = 4), inside of the science building (N = 2) and outside of the building (N = 4).

Effects of Sampling Date on Spider Diversity

Overall, we found a steady increase of species richness, spider abundance and Shannon-Wiener diversity from Lancer Park (i.e. outdoors habitat) from mid-March to late-April (Fig. 5). The spider diversity was relatively low in the habitats associated with outside of the science center building until mid-April; however, the diversity increased rapidly by

late-April with a similar abundance level compared to the Lancer Park. The spider diversity was relatively low and maintained at those low levels for habitats inside the science center building regardless of the sampling time (Fig. 5). However, the collective effects of sampling date across all habitats were not significant on species diversity (chi-squared = 4.0778, d.f. = 3, p-value = 0.2532), abundance (chi-squared = 4.4953, d.f. = 3, p-value = 0.2127) and Shannon-Wiener diversity (chi-squared = 2.3788, d.f. = 3, p-value = 0.4976), possibly due to small sample size.

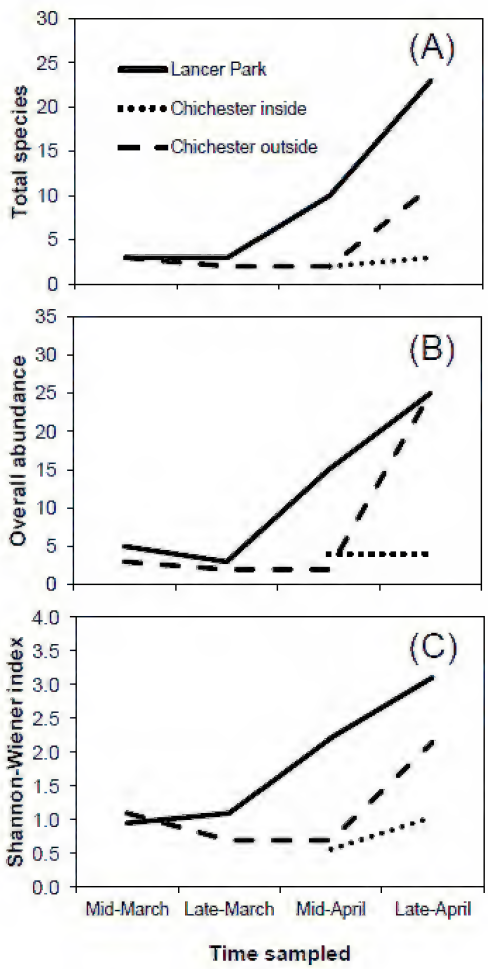

Figure 5. The effects of sampling time on number of species (A), overall abundance (B), and Shannon-Wiener diversity (C) reported from mid-March to late-April for three Sampling locations, Lancer Park (N = 4), inside of the science building (N = 2) and outside of the building (N = 4).

Effects of Environmental Conditions on Spider Diversity

We found a significant positive correlation between Shannon-Wiener diversity and temperature (R^2= 0.7092; $F_{1,8}$ = 22.95; P < 0.001) suggesting a significant increase of overall spider diversity as the temperature increases. However, we did not find any correlations between overall spider diversity and relative humidity (R^2= -0.1211; $F_{1,8}$ = 22.95; P > 0.05) suggesting no effect of relative humidity on spider diversity (Fig. 6).

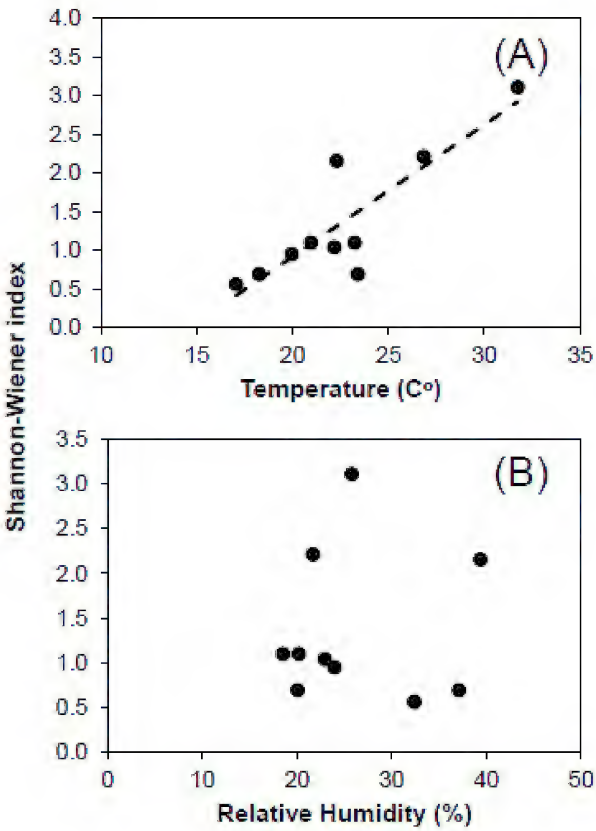

Figure 6. The effects of environmental conditions on spider diversity. A significant increase of overall spider diversity was observed as the temperature increases (R^2= 0.7092; $F_{1,8}$ = 22.95; P < 0.001), however no effect of relative humidity (R^2= -0.1211; $F_{1,8}$ = 22.95; P > 0.05). We have included both indoor (i.e. science building) and outdoor data for this analysis and each filled circle represents a single sampling event.

DISCUSSION

This preliminary survey of spiders along an outdoor-indoor habitat gradient has yielded an impressive 50 taxa of spiders (43 genera and 16 families) from a very limited study area, and with a relatively low sampling effort. The most abundant family was Araneidae (orb-weaver spiders) and the most abundant spider species was furrow orb-weaver (*Larinioides cornutus*; 9% of overall abundance). Additionally, wolf spiders (Lycosidae; 14.8%) and jumping spiders (Salticidae; 13.6%) also represented abundant groups. Spider species reported as only single specimens such as *Wulfila saltabundus* (Anyphaenidae), *Clubiona abboti* (Clubionidae) and *Drassyllus depressus* (Gnaphosidae) may represent rare taxa in the Piedmont region. Similarly, other regional studies that attempted to survey the diversity of spiders have yielded relatively higher values for the number of spider taxa with new state records (Mallis and Hurd 2005; Smith et al. 2018). This may indicate the hidden diversity of spiders in the eastern United States and emphasize the need for more research (also see Howell and Jenkins 2004) to document the full range of spider diversity covering a wide variety of habitats.

The highest diversity of spiders was reported from the outdoors habitats (i.e. Lancer Park = 36 taxa), followed by the marginal habitat (i.e. outside of the science building = 14 taxa), and the indoors habitats (i.e. inside the science building = 3 taxa). This may be due to habitat diversity together with the presence of varied microclimates in outdoors habitats compared to indoors and marginal habitats. Additionally, pest control within and around the Science Center using chemical agents may impact the spider diversity and abundance. Lancer Park is composed of various habitats including eastern deciduous forests, grasslands, hedge habitats, some buffer habitats (e.g. roads and parking lots) and aquatic habitats, which may create multiple niches to support diverse spider communities. Smith et al. (2018) also found a strong correlation between heterogeneity of habitats and spider diversity in Alabama. Upland xeric habitats with more plant diversity and moderate amounts of disturbance (i.e. fire) supported more spider diversity compared to less diversity in lowland ravine habitats. However, Mallis and Hurd (2005) found no relationship between the diversity of ground-dwelling spiders and successional age with various degrees of habitat complexity of six successional habitats in southwestern Virginia. Another possible reason for the observed correlation between spider diversity and habitat heterogeneity may be the diversity of prey base available for spiders. A more heterogeneous outdoor habitat would support a more diverse prey base compared to more homogenous indoor habitats, hence supporting a higher diversity of spiders in an outdoor habitat (Miyashita et al. 1998).

In general, urbanization poses a negative impact on species diversity and promote few urban-adapted taxa (Blair 1996). Therefore, one would expect less diversity of arthropods in indoor habitats compared to outdoor habitats. Although only a little attention has been given to the biodiversity of indoors by scientific communities, hundreds of arthropod taxa make human dwellings their home (Bertone et al. 2016) and have been co-existing with humans for thousands of years (Martin et al. 2015). Spiders are one of the major successful arthropod groups utilizing indoor environments including human dwellings (Bertone et al. 2016). However, to our knowledge, there are no reported studies comparing the spider diversity of indoor habitats with outdoor habitats. Therefore, our

study adds new information on indoor habitat use by spiders. Although we have observed marked increases of both number of species and abundance of spiders in outdoors and marginal habitats from mid-March to late-April, the indoor spider diversity was maintained at a low and constant level throughout the study period. The most abundant indoor spiders in our study were cellar spiders (*Pholcus* spp.). This may represent an indoor-adapted taxa since we did not find it from outdoor and marginal habitats, and it has a global distribution associated with human dwellings (Gaddy 2009). The lack of temporal variation of spider diversity in indoor habitats may be explained by the constant environmental conditions inside buildings (e.g. air temperature and humidity; Shochat et al. 2004), and very limited species diversity may be explained by the limited prey base and special adaptations needed to deal with urban environments (Blair 1996).

The temporal and spatial variation of spider diversity and abundance may be associated with variations in environmental conditions (Bolger et al. 2000; McIntyre et al. 2001) and spider life-history (Foelix 2011). We found a strong positive correlation between overall spider diversity and air temperature for outdoors and marginal habitats. Additionally, overall spider diversity and abundance have dramatically increased from mid-March to late-April, probably due to increased air temperature and hatching of egg sacs. McIntyre et al. (2001) also reported a positive correlation between spider diversity and abundance with air temperature. This may be due to more activity of spiders (and their prey) with increasing air temperature (Foelix 2011) and/or migration of spiders to suitable habitats tracking the increased temperature (Shochat et al. 2004). Although, some studies found a positive correlation between rainfall and spider diversity and abundance (Bolger et al. 2000), others reported no effect (McIntyre et al. 2001). We did not test the effects of rainfall, but we found that there was no effect of relative humidity on spider diversity.

Although limited in scope, our study adds original knowledge about the habitat use of spiders along an outdoor-indoor habitat gradient and arthropod use of the indoor biome. Additionally, our species occurrence data may be useful to update regional biodiversity inventories for the central Piedmont of Virginia. More importantly, this study adds novel information of potential impacts of urbanization on diversity and abundance of arthropods and stresses the need for more extensive studies to fully understand how spatial and temporal variation of arthropod diversity and abundance may be influenced by alterations of habitats by humans through urbanization.

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Henkanaththegedara designed the study, analyzed the data and wrote the manuscript. Kish collected the data and modified the manuscript. Authors thank Michael Pagliuca and Caitlin Harris for help with sample collection, Benjamin Campbell, Randy Durren, Caitlin Harris and two anonymous reviewers for reviewing an earlier version of this manuscript, and Longwood University Office of Student Research for funding. This study was conducted as an extension of Longwood BioBlitz (https://blogs.longwood.edu/longwoodbioblitz/) under VDGIF Scientific Collecting Permit # 059601.

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Appendix 1. The checklist of the spiders reported from the Lancer Park and Chichester Science Building at Longwood University.

Family	Common name	Scientific name
Anyphaenidae	Ghost Spider	*Wulfila saltabundus*
Araneidae	Arabesque Orbweaver	*Neoscona arabesca*
	Arrow-Shaped Micrathena	*Micrathena sigittata*
	Furrow Orbweaver	*Larinioides cornutus*
	Hackled Orbweaver	*Uloborus diversus*
	Humpback Orbweaver	*Eustala anastera*
	Marbled Orbweaver	*Araneus marmoreus*
	Orbweaver Sp.	*Larinioides sp.*
	Spotted Orbweaver	*Neoscona sp.*
	Star-Bellied Orbweaver	*Acantheperira stellata*
	Tuft-Legged Orbweaver	*Mangora placida*
Clubionidae	Leaf-Curling Sac Spider	*Clubiona abboti*
Gnaphosidae	Ground Spider	*Drassyllus depressus*
Linyphiidae	Filmy Dome Spider	*Neriene radiata*
	Black-Tailed Red Sheetweaver	*Florinda coccinea*
	Bowl and Doily Spider	*Frontinella pyramitela*
Lycosidae	Carolina Wolf Spider	*Hogna carolinensis*
	Pirate Wolf Spider	*Pirata sp.*
	Rabid Wolf Spider	*Rabidosa ribida*
	Thin-Legged Wolf Spider	*Paedosa sp.*
	Wolf Spider Sp.	*Alopecosa aculeata*
Oxyopidae	Lynx Spider Sp.	*Oxyopes aglossus*
	Striped Lynx Spider	*Oxyopes salticus*
Philodromidae	Running Crab Spider	*Philodromus placidus*
	Running Crab Spider	*Philodromus sp.*
Pholcidae	Cellar Spider	*Pholcus manueli*
	Long-Bodied Cellar Spider	*Pholcus phalangioides*
Pisauridae	Dark Fishing Spider	*Dolomedes scriptus*
	Nursery Web Spider	*Pisaurina mira*
	White-Banded Fishing Spider	*Dolomedes albineus*
Salticidae	Bold Jumper	*Phidippus audax*
	Bronze Jumper	*Eris militaris*
	Flat Jumper	*Platycryptus undatus*
	Golden Jumper	*Paraphidippus aurantius*
	Jumping Spider Sp.	*Phidippus sp.*
	Jumping Spider Sp.	*Tutelina elegans*
	Jumping Spider Sp.	*Sitticus ammophilus*
	Jumping Spider Sp.	*Naphrys pulex*
	Zebra Jumping Spider	*Salticus scenicus*

Tetragnathidae	Long-Jawed Orbweaver	*Tetragnatha straminea*
	Orchard Orbweaver	*Leucage venusta*
Theridiidae	Common House Spider	*Parasteatoda tepidariorum*
	False Black Widow	*Steatoda grossa*
	Cobweb Spider Sp.	*Theridula opulenta*
	Cobweb Spider Sp.	*Neospintharus trigonum*
Thomisidae	Elegant Crab Spider	*Xysticus elegans*
	Ground Crab Spider	*Xysticus ferox*
	Tricolored Crab Spider	*Synema parvulum*
Uloboridae	Hackled Orbweaver	*Uloborus glomosus*

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Mercury Concentrations in Bat Guano from Caves and Bat Houses in Florida and Georgia

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ABSTRACT

Previous studies have indicated that several pollutants are bioaccumulating in insectivorous bats, including the heavy metal mercury. This has resulted in an increased presence of mercury in bat waste (guano). In this study, we collected bat guano from ten caves in Florida and Georgia and two bat houses in Florida and analyzed the samples for mercury concentrations (ppm). Since the predominant bat species using caves (*Myotis austroriparius)* versus bat houses (*Tadarida braziliensis*) were different, the objective of this study was to make statistical comparisons of the mercury concentrations among caves, between caves and bat houses, and between bat houses. We found no significant differences between caves and bat houses. The mean concentrations among caves were significantly different, as well as the concentrations between the two bat houses. These results show similar levels of mercury concentrations in bat guano in both predominant bat species that use these caves and bat houses in Florida and Georgia. But variability exists between all locations, which indicate that other variables (e.g., geographic hot spots for mercury exposure) also affect mercury concentrations in guano. This study provides baseline data for bat guano mercury levels, which is a barometer of bat health and potential bioaccumulation of mercury in guanitic food webs, such as those in cave ecosystems.

Key words: Bat guano, mercury, insectivorous bats, bioaccumulation

INTRODUCTION

Elemental mercury can transform in aquatic systems by bacterial methylation to methylmercury, a neurotoxin which bioaccumulates in aquatic and terrestrial food webs (Brasso and Cristol, 2008; Selin, 2009). Insectivorous bats are particularly susceptible to mercury bioaccumulation via trophic transfer (Iskali and Zhang, 2015; Syaripuddin et al.,

2014). They take up mercury when consuming large quantities of insects that accumulate mercury during their aquatic larval stages in mercury-contaminated waterbodies, as well as when feeding on terrestrial insects that bioaccumulate mercury (Brack and Whitaker, 2001; Becker et al., 2017).

Mercury in the environment is unquestionably affecting bats, since several studies have linked mercury pollution and the presence of mercury in various body parts of bats (Powell, 1983; O'Shea et al., 2001; Wada et al., 2010; Syaripuddin et al., 2014). This heavy metal contamination is linked to bat population declines (Mickleburgh et al., 2002) and sub-lethal biological effects like impaired reproduction and chronic health issues, as well as death in bats exposed to high contaminant loads of heavy metals (Clark and Shore, 2001; Hickey et al., 2001). Bats with white-nose syndrome have been found with elevated levels of contaminants and mercury exposure that potentially predisposes bats to this disease (Kannan et al., 2010).

Part of the mercury load in bats is excreted in their fecal matter, called guano, which primarily consists of bat hair, insect remains and bat mucus (Maher, 2006). Guano can therefore be examined for the presence of mercury in a habitat or ecosystem that contain bats, such as a cave or bat house. Several studies have already shown the presence of mercury in bat guano from caves. Petit and Altenbach (1973) dated a guano core from a cave in Colorado and found levels of mercury throughout the core were related to production at a local copper smelter and open pit mine. O'Shea et al. (2001) found higher concentrations of environmental contaminants, including mercury, in bat guano near a superfund site than at a reference site in Colorado. Petit (1975) investigated mercury concentrations in a 1100 year-old guano core from an Arizona cave and suggested that mercury concentrations had been higher than expected in pre-industrial times, possibly due to geological processes such as volcanic activity. A recent study by Hagan (2014) analyzed three age-dated groups of bat guano from Mammoth Cave National Park in Kentucky and found that modern/fresh guano had higher concentrations of mercury than historical guano (~100-1100 years old), which in turn had higher concentrations than ancient guano (~30,000 years old).

The concentration of mercury in bat guano has implications not just for the health of bats, but also cave ecosystems. The presence of mercury in guano allows potential for mercury to bioaccumulate in guanitic food webs for trogloxenes, troglophiles and troglobites. Coprophagy of guano is observed in cave-adapted salamanders (Fenolio et al., 2006), dermestid cave beetles (Mizutani et al., 1992), and even meat ants who enter caves to collect and transport guano back outside to their mounds (Moulds, 2006). Macroinvertebrate communities in caves increase after fresh guano is deposited (Poulson and Lavoie, 2000), and the nutrient quality of guano influences biodiversity of macroinvertebrates in caves (Iskali and Zhang, 2015).

This study had two objectives: one, to collect data on total mercury concentrations (ppm) in bat guano from two major bat ecosystems (ten caves and two bat houses) in a geographic region with documented inorganic mercury loading via atmospheric deposition from local and global sources (Stephenson, 1997; Prestbo and Gay, 2009), and abundant

waterbodies to methylate the mercury; and two, to use the data to make statistical comparisons between caves and bat houses. Our first hypothesis predicted that since similar species roost in all caves in the study, then the concentrations of mercury in guano from all caves would be similar. Our second hypothesis predicated that the since the dominant species of bats differs between caves (*Myotis austroriparius*) and bat houses (*Tadarida braziliensis*), then concentrations of mercury in guano would be significantly different between these types of dwellings. Data resulting from this study are a valuable baseline for future studies to monitor the potential mercury contamination in bats and cave ecosystems in Florida and Georgia.

MATERIALS AND METHODS

The guano from insectivorous bats in this study was collected from eight caves and two bat houses in Florida and two caves in southwestern Georgia (Figure 1), depicted using the R package ggmap (Kahle and Wickham, 2013). The total number of guano samples we collected from caves was 95, and 17 for bat houses. All samples were collected between January 12, 2013 and February 13, 2014. Due to the heterogeneous nature of the quantity and depth of guano available in the caves sampled, the number of samples collected from each cave were not the same.

Both core and surface samples were collected for the study. Core samples were collected with a Russian sampler to avoid compaction of guano (Maher, 2006, Johnston et al., 2010). Cores were divided into 1 inch (25.4 mm) subsamples starting from the top of the core. Guano samples from cave surfaces were collected with plastic spoons and put into clear, reclosable plastic bags, with a new spoon and bag for each sample. Detailed information on guano samples is presented in Table 1. Sample locations were estimated on cave survey maps and may be requested from the corresponding author.

The dominant bat species roosting in Florida and Georgia caves are the maternity/wintering colonies of the Southeastern myotis (*Myotis austroriparius*) (Gore and Hovis, 1998), with lesser contributions from Tri-colored bats (*Perimyotis subflavus* - formerly known as eastern pipistrelle, *Pipistrellus subflavus*). The endangered Gray bat (*Myotis grisescens*) was formerly abundant in some caves in the Florida, but the Florida population has decreased in the last few decades and the species may no longer be present in the state (Gore et al., 2012). The caves in Georgia have *Myotis austroriparius* and *Perimyotis subflavus* as the dominant species (pers. comm. K. Morris, Georgia Department of Natural Resources, April 24, 2013). Thus, we assumed that *Myotis austroriparius* is the dominant species contributing to the guano piles in caves in this study. M. *austroripariu* forage near water (Barbour and Davis, 1969) and consume arthropods, (primarily Coleptera, Lepidoptera and Culicidae (Zinn, 1977). *Perimyotis subflavus* also forage near water (Fujita and Kunz, 1984; Whitaker and Hamilton, 1998) and consume insects in the orders of Trichoptera, Homoptera, Coleoptera, Hymenoptera and Lepidoptera (Sherman, 1939; Ross, 1961; Whitaker, 1972; Carter et al., 1999).

Guano samples were taken below two bat houses in central Florida, detailed in Table 1. The dominant species roosting in bat houses in Florida is the Brazilian free-tailed bat (*Tadarida braziliensis*), with *Myotis austroriparius* present to a lesser degree. In the

southeastern United States, the *Tadarida braziliensis* diet includes insects in the order of Coleoptera, Diptera, Lepidoptera and Hymenoptera (Sherman, 1939).

All samples were stored in a freezer until they were freeze dried to constant weight and analyzed for Total Mercury (THg) by thermal decomposition, gold amalgamation and atomic absorption spectroscopy (EPA method 7473) using a Milestone DMA80 mercury analyzer (Milestone, Inc., Shelton CT). This analyzer has a detection limit as low as 0.001 nanograms of mercury and as high as 300 ppm (mg/kg). The QA/QC included blanks, replicates and matrix spikes. All replicates had <10 percent difference and were averaged. The DMA80 was calibrated with NIST-traceable standards. Quality assurance included standard reference materials with similar total Hg concentrations purchased from NIST and the National Research Council of Canada. Results are reported as total mercury per gram dry weight of guano. The open-source statistical computing package R (R Core Team, 2018) was used to make graphics and conduct statistical analyses.

RESULTS

A total of ten caves were surveyed during the data collection phase of this project. However, in four of these caves, we were able to collect fewer than three samples. This is too little information to reliably ascertain the mean of mercury concentrations in these four caves, so we excluded these caves from our comparison of mean mercury concentrations at the various locations. The left panel of Figure 2 shows the concentrations from the remaining six caves, along with the number of observations available for each.

For the samples which came from cross-sections of guano cores, we investigated whether there was evidence indicating whether mercury concentration tended to increase or decrease with depth (distance in inches from the top of the core). We also checked for serial correlation between the layers of each core. Only in the case of one core, taken from Judge's Cave, was there any evidence of statistically significant association between mercury concentration and depth or statistically significant autocorrelation. However, the subsamples from the other core taken within Judge's Cave did not conform to this pattern. We suspect that the seeming significance of this result may have been due to type I error, given the lack of corroboration with the other core taken from the same cave. With only eight guano cores of varying sizes, containing 6-11 subsamples each, we are limited in our ability to detect meaningful relationships. In addition, we note that the caves are subject to frequent flooding events, which may affect the mercury concentrations within the guano cores.

The Fligner-Killeen test of homogeneity of variances (Fligner and Killeen, 1976) indicated that variances in mercury concentrations among the six caves cannot be assumed to be equal ($p < 0.001$). Therefore, we cannot compare the mean concentrations among the caves using the traditional one-way analysis of variance (ANOVA) approach, since ANOVA assumes homogeneity of variances. Instead, we use generalized least squares (e.g. Sen and Srivastava, 1990, Chp. 6) to estimate a mean concentration for each cave, while also accommodating the differing variability among the caves.

To facilitate the comparison of all pairs of caves, we used the emmeans package in R (Lenth, 2018) and applied Tukey's adjustment for multiple comparisons. We found significant differences in mean mercury concentrations between Climax Cave and Florida Caverns Old Indian Cave ($p < 0.001$) and between Climax Cave and Judge's Cave ($p < 0.001$). The mean mercury concentration in Florida Caverns Old Indian Cave is estimated to be 0.20 +/- 0.08 ppm higher than that in Climax Cave, while the mean in Judge's Cave is estimated to be 0.21 +/- 0.10 ppm higher. Using the estimated cave mean concentrations and variances, we can also derive a confidence interval for the overall mean concentration of mercury across the caves, which is 0.55 +/- 0.05 ppm.

The right panel of Figure 2 depicts the mercury concentrations in the samples obtained from the two bat houses. Given the small number of samples taken from the Lower Suwanee National Wildlife Reserve (NWR) Bat House, it is difficult to assess the variability of mercury concentrations, and we cannot assume that the population variances of the two groups are equal. Using the generalized least squares technique, as we did for the caves, the mean concentrations in the two bat houses were found to be significantly different ($p < 0.001$). The mean concentration at the bat house at the Lower Suwanee NWR was estimated as 0.37 +/- 0.14 ppm higher than the concentration at the bat house at the University of Florida at Gainesville.

In the sample sizes provided in Figure 2, we note that the samples available from the two bat houses were limited, both in sample size and in the number of bat houses observed. Given the smaller sample sizes for the bat houses, especially for the one at the Lower Suwanee NWR, the validity of the normal distribution assumed by our generalized least squares model can be reasonably be questioned. However, the Wilcoxon-Mann-Whitney non-parametric test procedure, which does not depend on the normality assumption, provides additional evidence that the concentrations are significantly different between the two bat houses ($p < 0.001$).

Using the estimated means and variances from the generalized least squares model, as we did for the caves, we estimate the average mercury concentration for the two bat houses as 0.51 +/- 0.05. Since this confidence interval overlaps the confidence interval we developed for mercury concentrations in caves, we do not have enough evidence to say that the mean concentration levels are significantly different between the cave-dwelling and bat house-dwelling bats we observed. We note that this aspect of the analysis is limited by the number of bat houses that could feasibly be sampled; the addition of observations from other bat house locations would improve our estimates.

DISCUSSION

In the first hypothesis, we predicted that, since similar species roost in all the caves sampled in this study, the concentrations of mercury in guano among caves would be similar. Data failed to support this hypothesis, as there was a significant difference of mean concentrations between caves. Given that the caves play host to the same predominating species of bat, any significant differences in mean mercury concentrations among locations is a possible indicator of differing pollutant levels in the surrounding environment. Variations could be due to geographic hot spots of mercury pollution, since a major source

of mercury pollution in Florida and Georgia is atmospheric deposition. There could also be other factors that potentially affect mercury bioaccumulation in bats including local differences in available prey types, and possible differences in colony sex ratios, animal sizes or population age structure, which would affect the bat colonies and their guano in the caves.

Our second hypothesis was also not supported by the data comparing cave and bat houses with different predominant species, as the mercury concentrations in guano were not significantly different between caves and bat houses. This would indicate that mercury concentrations in guano from both predominant species in caves (*Myotis austroriparius*) and bat houses (*Tadarida braziliensis*) were similar.

It is interesting to note that the estimated mean concentrations of mercury in bat guano in both caves and bat houses in this study area of Florida and Georgia lies within the mean range of the modern/fresh guano (0.7 +/- 0.2 ppm) from the Hagan 2014 study in Kentucky. The guano collected for this study was assumed modern (<100 years old, as in the Hagan, 2014 study), since none of the guano produced cores over 11 inches (279.4 millimeters), indicating a lack of long-term accumulation of guano piles. The guano piles from the bat houses were also known to be disturbed. The guano at the University of Florida, Gainesville bat houses are collected in 55 gallon drums and given away in 5 gallon buckets to gardeners several times per week (Kenneth Glover, per comm, 4/26/2013).

Analysis would be improved by increasing the number of samples from bat houses. This would include increasing the sample size from each bat house, particularly as we had only 5 samples from the Lower Suwanee NWR bat house, or increasing the number of bat houses from which samples were obtained. The Lower Suwanee bat house is a much smaller bat house than the bat house at University of Florida at Gainesville, which has the largest occupied bat house in the world (https://www.floridamuseum.ufl.edu/bats/) and two structures for bat houses instead of the one structure at Lower Suwanee.

Other weaknesses of the study include the low sample size and the differences in the age of the guano among locations, possibly skewing the results. The two bat houses in this study are known to give away guano on a routine basis to gardeners, thereby removing the older guano. Although guano in cave environments are disturbed by cavers and cave scientists, caves are a more protected environment from the elements than bat houses, so the guano from caves in this study are likely older and less disturbed than the guano under the bat houses. The effect of flooding on guano piles in regards to mercury mobility is also unknown, as is bioturbation from fauna.

Future studies should evaluate methylmercury concentrations in both fresh guano and hair from individual bats to correlate concentrations between the bats and the bat waste. It would also be beneficial to know if the bacteria that convert inorganic forms of mercury to methylmercury existed in caves, as the methylmercury is the bioavailable form. A study of mercury mobility in guano would also be interesting, as our study found a lack of significant difference in variances among the core and surface samples, as well as samples from the same core. Data resulting from this study are a valuable baseline for future studies

to monitor the potential mercury contamination in bats and cave ecosystems in Florida and Georgia.

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Figure 1: Location Map

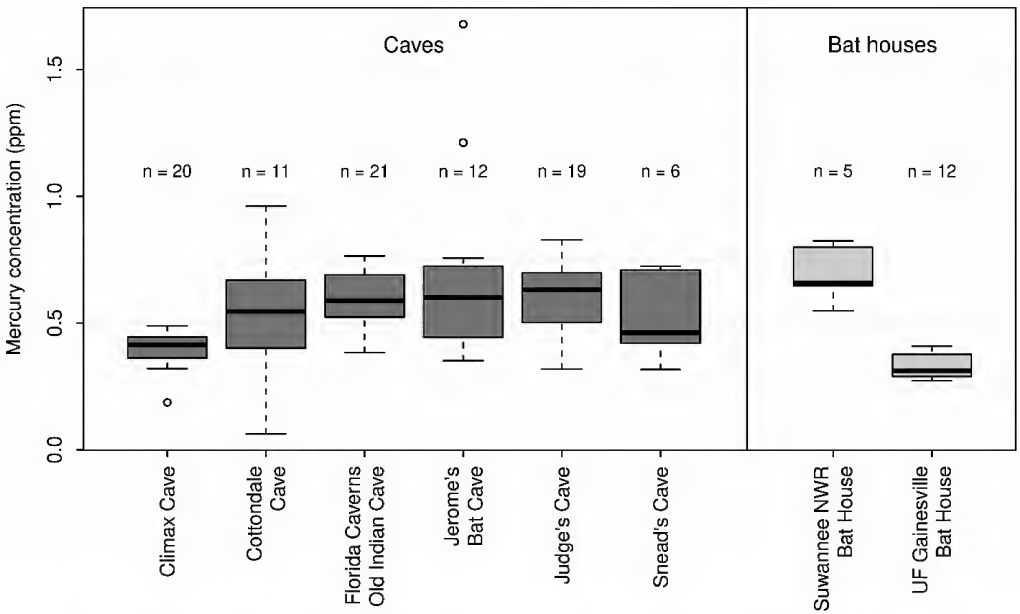

Figure 2: Boxplots of mercury concentrations, caves versus bat houses

Table 1: Guano samples from caves and bat houses

Location	State	Date collected	Sample details
Big Mouth Cave	Florida	7/13/2013	bat pellets collected throughout cave and compiled into 1 sample
Cottondale Cave	Florida	10/30/2013	5 surface samples taken throughout the cave and a core that was 6 in (152.4 mm) deep and subsampled at 1 in (25.4 mm) intervals
Florida Caverns Old Indian Cave	Florida	2/13/2014	two 8 in (203.2 millimeters) cores subsampled at 1 in (25.4 mm) intervals in the Rotunda Room, 5 surface samples taken throughout cave
Jerome's Bat Cave	Florida	10/30/2013	12 surface samples taken throughout cave
Judge's Cave	Florida	10/30/2013	one 11 in (279.4 mm) core subsampled at 1 in (25.4 mm) intervals, and one 8 (203.2 mm) core at 1 in (25.4 mm) intervals
Newberry Bat Cave	Florida	9/15/2013	1 sample of bat pellets
Snead's, also known as Pope's Bat Cave	Florida	10/30/2013	6 surface samples
Thornton's Cave, also known as Sumter Bat Cave	Florida	7/13/2017	1 sample at one of the entrances
Climax Cave	Georgia	2/28/2013	3 surface samples from the Barrel Room
Climax Cave	Georgia	7/6/2013	10 in (254 mm) core subsampled at 1 in (25.4 mm) intervals from Barrel Room, 4 surface samples, 2 composites of 2 separate 11 in (279.4 mm) cores, two separate samples that were the bottom inches of two separate 11 in (279.4 mm) cores
Waterfall Cave	Georgia	8/17/2013	2 surface samples
University of Florida bat house	Florida	9/13/2013	One core had 5 intervals of 1 in (25.4 mm) each. The other core had 7 intervals, with the first six intervals at 1 in (25.4 mm) and the 7th interval comprising the last 1.5 inches of the core.
Lower Suwanee National Wildlife Reserve	Florida	1/26/2014	The guano piles were not deep enough to use the corer, so samples were taken as compilations within

			different locations under the bat house. Depths were measured in inches with a ruler from the top of the pile to the concrete bottom. A 4 in (101.6 mm) sample was compiled from the middle under the bat house, a 3 in (76.2 mm) sample was compiled from the back left corner, a top inch (25.4 mm) was taken from the back middle, and the bottom inch (25.4 mm) was taken from the back middle.

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Stream Assessment on the Impact of Agricultural Activity in the Dry River, VA

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ABSTRACT

Stream bioassessments using macroinvertebrate population dynamics is a technique that determines water quality in natural aquatic environments based on the taxa found at the site. The aim of this study is to determine if agricultural activity in Rockingham County, VA has an impact on water quality in Dry River. Stream quality was evaluated by sampling and identifying macroinvertebrate taxa at various sites above and below disturbances. Each macroinvertebrate was ranked from 1-10 based on pollution tolerance or intolerance using the Biological Monitoring Working Party Index. The results in this study indicate that agricultural activity does impact the water quality in Dry River in Virginia.

Keyword: macroinvertebrates, agriculture, pollution, Dry River, stream quality

INTRODUCTION

The Dry River begins in the George Washington National Forest and flows through the Shenandoah Valley in Southwest Virginia. It is home of many aquatic and terrestrial organisms including fish and bird species that use the Dry River habitat. Dry River provides a great trout fishery in Virginia and holds rainbow, brown, and brook trout (Authors personal observations). The Virginia Department of Game and Inland Fisheries stock various locations throughout Dry River (VDGIF stocking website) to facilitate this recreational fishery. Migrating birds also use Dry River Valley. Therefore, Dry River becomes a recreational opportunity that adds ecotourism opportunities that create revenue for the small towns and cities that run its length. Rockingham County, which Dry River flows through, is the leading poultry-producing county in Virginia (Bosch and Napit 1992). Along with poultry production in Virginia, Rockingham County is the leading producer of corn silage, dairy cattle, hay, alfalfa, and ranks the highest in farm income (Pease and Kenyon, 1992). The high percentage of land use in agriculture that surrounds the Dry River could affect water quality and environmental integrity.

In the last 40 years, antipollution laws have reduced discharge of point source pollution of toxic substances into freshwater (Howarth et al., 2000). However, less effort has been made to restrict non-point source pollution of nitrogen (N) and phosphorus (P) that enter freshwater from agricultural and urban runoff (Howarth et al., 2000). Agriculture can affect aquatic ecosystems through the run-off of fertilizers, manure, and pesticide applications (Pease and Kenyon, 1992). Livestock can overgraze riparian areas, creating a loss of stability to streambanks, which causes soil erosion, and overall declining water quality (Belsky et al., 1999). Inputs of nonpoint pollutants from agriculture have increased dramatically and now N and P represents the largest pollution problem facing freshwater as well as the coastal waters (Howarth et al., 2000). Consequently, agriculture can impact natural aquatic ecosystems negatively and ecosystems can become biologically imbalanced (Moss, 2008).

Nutrient over-enrichment of aquatic ecosystems can trigger ecological imbalance that decrease the biological diversity (Howarth et al., 2000).This imbalance can affect aquatic species which has caused fish kills and advisories in neighboring rivers such as the Shenandoah, New, and Roanoke Rivers. During the past couple decades research has found phosphorus to be the biggest driver of eutrophication of freshwater systems (Howarth et al., 2000). High levels of nitrogen and phosphorus in the water harm both vertebrate species and invertebrate species. Fish that are in contact with high nutrient levels affect cardiovascular processes, behavior, endocrine system, and excretory processes (Kuklina et al., 2013). Invertebrate species, when living in a high nutrient environment, are affected through the loss of locomotive abilities, cardiac distress, and unusual behavior (Kuklina et al., 2013).

Plant communities are also impacted by nutrient additions. Aquatic plant communities are the basis for a healthy and diverse aquatic ecosystem, providing food, shelter, and breeding habitats for aquatic species (Withers and Lord 2002; Mainstone and Parr 2002). Nutrient enrichment in freshwater systems can degrade plant community by altering the competitive balance between different aquatic plant species (Mainstone and Parr 2002). Diffuse sources of phosphorus, particularly from agriculture, are a major contributor to phosphorus levels in riverine sediments, where it can be utilized by benthic algae and rooted plants. This phosphorus can also be released into the water column by a variety of processes (Mainstone and Parr 2002).

Testing water quality can be completed using equipment to measure pH, turbidity, nitrates, phosphates, and dissolved oxygen (Kuklina et al., 2013). Water quality can also be tested with bioindicator species such as macroinvertebrates due to their sensitivity to pollutants. Bioindicator species are often used because macroinvertebrates are a good indicator of the cumulative effects of pollution (Lenat, 1984). In a study of the Ontario stream system (Marsh and Waters, 1980), two branches were surveyed for macroinvertebrates. There was a branch with high agricultural land use in the drainage and a branch with no agricultural land use in the drainage. They found that the branch with high input of agricultural drainage showed a decrease in taxa richness of intolerant groups (*Plecoptera*, *Ephemeroptera*, and *Trichoptera*) and an increase in taxa richness of tolerant groups (*Coleoptera*, *Odonata*). The presence and tolerance level of macroinvertebrates

showed that agricultural drainage from nearby farms was negatively affecting the Ontario stream's water quality (Marsh and Waters, 1980).

The goal of this study is to investigate whether water quality in the Dry River is being affected by the agricultural activity. It is important to have water quality assessment to determine water impairment of an aquatic environment which potentially needs management, alternative forms of fertilization, and/or different methods of soil treatment. A healthy stream is vital for both health and economic usage of the waterway, which boosts local and state economies through consumer interaction and licensing.

METHODS

Macroinvertebrates were sampled in the Dry River located in Rockingham County, VA. The Dry River starts from Skidmore Lake located in the George Washington and Jefferson National Forests and ends entering the North River in Dayton, VA. Macroinvertebrates were sampled along US. Route 33 at Riven Rock State Park (Location AA) and in Dayton, 100 meters above the junction to the North River (Location BA). Location AA exists above agricultural activities including dairy and beef cattle farming, poultry broilers and layers, and corn and soybean fields. Location BA is located below these agricultural activities.

Sampling was conducted between April and June of 2017 using the Surber sampling method. Location AA and BA were sampled once a day in April, May, and June. Each sampling day consisted of three isolated locations within sites AA and BA. Within the river the sampling was performed in fast flowing water and a usable substrate for Surber sampling purposes. At each of the sampling points (3 within AA and BA) three Surber samples were taken and combined for each point location (3) within AA and BA. The sampler was firmly placed in the substrate and the substrate was disturbed. The duration of disturbance varied based on substrate characteristics. The goal was to obtain all macroinvertebrates within the sampling area. Each macroinvertebrate was then carefully placed in a 33cm X 21cm aluminum pan and identified to the lowest taxonomical level possible. Each individual was identified using the macroinvertebrate key created by Birmingham et al., (2005).

These data were analyzed using the modified version of the Biological Monitoring Working Party index (BMWP) (Uherek and Gouveia, 2014). The BMWP was used to score taxa from 1-10 and the respective scores were used to determine Average Score Per Taxa (ASPT) at their respective sample locations. To get the score, each organism is given a number according to the BMWP scoring system (Uherek and Gouveia, 2014; See Appendix 1). A total score is calculated for each site and divided by the number of species to obtain the ASPT. Percent EPT was also used to provide another data point to indicate aquatic system health. EPT can be expressed as a percentage of the sensitive orders (E= *Ephemeroptera*, P= *Plecoptera*, T= *Tricoptera*) to the total taxa found. A large percentage of EPT taxa indicates high water quality. The ASPT results (dependent variable) and percent EPT (dependent variable) were analyzed to the differences between sites (independent variable). Shapiro-Wilk test was performed to test normality in the data before the statistical analysis. The ASPT scores were found to be normal and analyzed for

significance using a t-test assuming unequal variance. Percent EPT for sites were compared using z-test statistics (Uherek and Gouveia, 2014).

RESULTS

Sample results included 258 individuals belonging to 11 different taxonomic groups. Table 1 depicts species order and relative abundance at each location. According to the scoring parameters of the BMWP, above agricultural activity sites (AA) scored a 48 and below agricultural activity sites (BA) scored a 47 (Table 3). In the study by Uherek and Gouveia (2014) these totals were used to characterize water quality. Table 2 presents data on stream quality based on overall score from taxa. According to Table 2, each site would be considered Class III with scores of 48 (AA) and 47 (BA) falling between the 36-60 BMWP score range. This classification indicates that water quality is questionable and has been moderately impacted. According to this ranking system used by Uherek and Gouveia (2014), Location AA and BA are not significantly different in water quality characteristics. However, this study and sample sites did not produce large numbers in species abundance or species richness. Therefore, statistical importance can be shifted to an ASPT statistic and analysis conducted to further investigate significant difference between each site.

The BMWP scoring parameters were used to determine ASPT. The ASPT for AA was 8 and 5.2 for BA. There is a significant difference in ASPT between the two primary test sites (p-value=0.039, T = 1.89) (Figure 1).

AA sites had 75 individuals that belonged to EPT taxa and BA sites respectively had 24 individuals. Percent EPT for AA was 62.5% and 17.4% for BA. There was a significant difference between these percentages ($z=7.43$, $\alpha=1.96$, $z>1.96$) (Figure 2). According to these results, there is a significant difference between water quality above agricultural activity and below agricultural activity in the Dry River, VA.

DISCUSSION

According to this study, agricultural activity does influence water quality in the Dry River. The relative water quality of above agricultural sites (AA) were better that of below agricultural sites (AA) indicating a negative impact of agricultural activities. There is a plethora of research that supports the notion of negative impacts of agricultural activities in river pollution (Strokal et al., 2016; Howarth et al., 2000; Ribbe et al., 2008; Mainstone and Parr 2002). Some of the major problems with agricultural pollution is the identification of sources and the impacts of agricultural pollution (Ongley, Xiaolan, and Tao 2010; Quan and Yan 2002; Shortle and Abler 2001; Chambers and Quiggin 1996). Restricting non-point source pollution is very difficult because it is hard to identify the source of the pollution (Howarth et al., 2000). The agricultural pollution usually comes in the form of nutrient pollution (Howarth et al., 2000; Ongley, Xiaolan, and Tao, 2010). However, the amount of nutrients from each farm varies based on the season and location (Liu, Wu, and Zhang, 2005). This variation impacts biota in different ways and identification of those impacts are challenging (Howarth et al., 2000).

The importance of water quality bioassessment begins with the health and safety of individuals and the affected environment. The results show agriculture influences water

quality; however, the severity is not fully understood. Future studies would need to be performed to uncover the potential impacts and the temporal and spatial variation of such impacts. The impact on water quality, due to agriculture, could potentially affect not only macroinvertebrates but also other species in the food web such as fish and birds. This could affect the local economy by a decrease in fisherman and ecotourism. Farmers and landowners should be made aware of the impacts to water quality for hopes that pristine or better conditions may be obtained.

Further research should be focused on discovering what pollution is in the stream that is affecting macroinvertebrates. Macroinvertebrates are a great bioindicator (Birmingham et al., 2005; Hilsenhoff, 1988), but they do not tell us what impacts are occurring. This would provide insight on the severity of water quality impacts by agricultural disturbances. Once the chemical composition is obtained, the direct source could be identified, and pollutants potentially minimized.

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Table 1. Sampling results from each location showing common name, species taxonomic order, and abundance.

Dry River (above AD)	Order	Species Richness
Caddisfly	*Trichoptera*	30
Stonefly	*Lecoptera*	36
Helgramite	*Megaloptera*	15
Damselfly	*Odonata*	19
Mayfly	*Ephemeroptera*	9
Crawfish	*Decapoda*	11
	Total	120

Dry River (below AD)	*Order*	Species Richness
Aquatic Worm	*Oligochaeta*	28
Crayfish	*Decapoda*	8
Water Penny	*Coleoptera*	23
Caddisfly	*Trichoptera*	11
Midge	*Diptera*	32
Stonefly	*Plecoptera*	13
Helgramite	*Megaloptera*	5
Snail	*Mollusca*	16
Backswimmer	*Notonectidae*	2
	Total	138

Table 2. Water quality interpretation table according to Uherek and Gouveia (2014). Site AA shows a score of 48 and site BA shows a score of 47. Each site would be considered class III meaning water quality is moderately impacted (Uherek and Gouveia 2014).

Class	BMWP score	Category	Interpretation
I	101-150, >150	Good	Very clean water and not significantly impacted
II	61-100	Acceptable	Clean but slightly impacted
III	36-60	Questionable	Moderately impacted
IV	15-35	Critical	Polluted or impacted
V	<15	Very Critical	Heavily polluted

Table 3. This table depicts all sites samples at AA and BA. BMWP totals are depicted on the right and each X indicates if the species was present at the location (Uherek and Gouveia, 2014).

Location (AA)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Points**
Coleoptera										
Decapoda		X	X	X		X		X		6
Diptera										
Ephemeroptera	X		X	X			X			10
Megaloptera		X	X		X	X	X	X	X	4
Mollusca										
Notonectidae										
Odonata	X		X	X	X	X			X	8
Oligochaeta										
Plecoptera	X	X	X	X	X		X	X	X	10
Trichoptera	X	X	X	X	X	X	X	X	X	10
									TOTAL	**48**
									ASPT	**8**
Location (Below AG)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	
Coleoptera	X		X		X	X	X		X	5
Decapoda		X		X		X		X		6
Diptera	X	X	X	X	X	X	X	X	X	5
Ephemeroptera										
Megaloptera	X		X		X					4
Mollusca		X	X	X	X					3
Notonectidae				X						3
Odonata										
Oligochaeta	X	X	X	X		X	X	X	X	1
Plecoptera			X			X		X		10
Trichoptera			X				X		X	10
									TOTAL	**47**
									ASPT	**5.2**

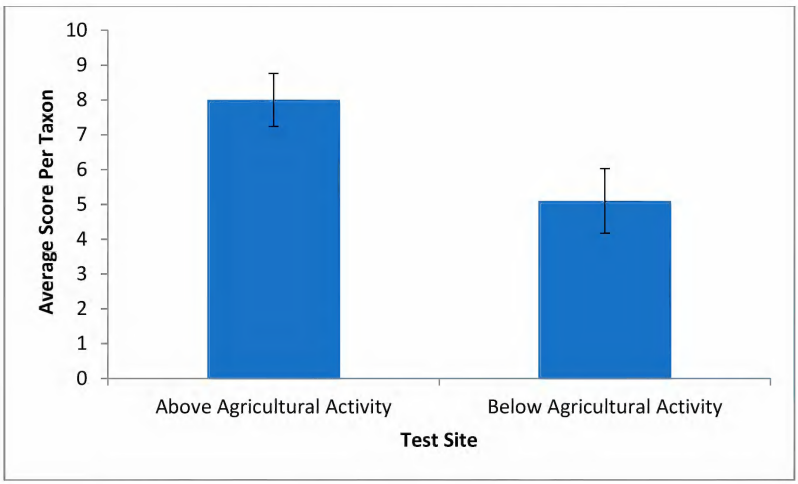

Figure 1. Average score per taxa (ASPT) between the two primary sample sites: Above agricultural activity (AA) (AA=8, SE=0.76), below agricultural activity (BA) (BA=5.2, SE= 0.93) (p-value=0.039, T = 1.89).

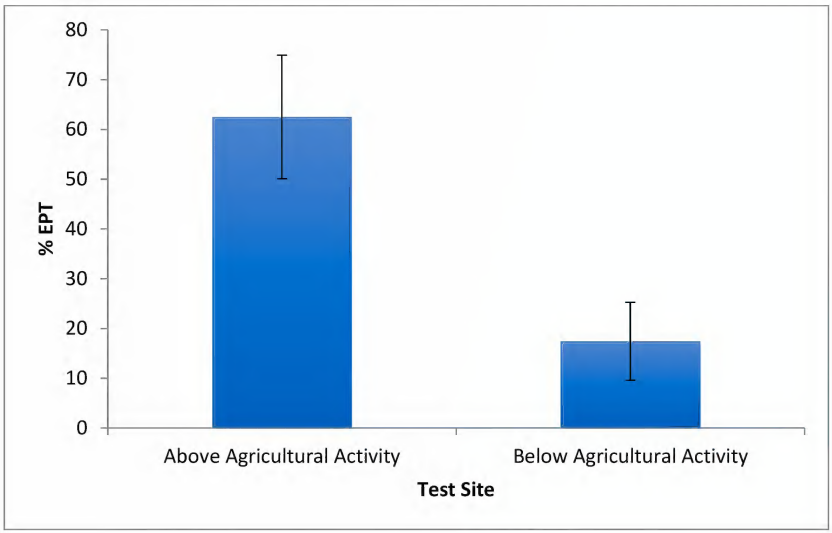

Figure 2. Percent EPT at each test site. Test site AA %EPT=62.5, SE=12.43, Test site BA %EPT=17.4, SE=7.83, Statistical analysis (z=7.43, α=1.96, z>1.96).

Appendix 1

Table. Scoring Index used from the modified version of the Biological Monitoring Working Party (BMWP) from Uherek and Gouveia (2014).

Taxa	Score
Ephemeroptera: *Leptophlebidae, Leptohyphidae.* ***Plecoptera:*** *Perlidae* ***Trochoptera:*** *Brachycentridae, Leptoceridae, Odontoceridae, and Sericostomatidae.*	10
Odonata: *Coenagrionidae, Calopterygidae, Gomphidae, and Libellulidae.* ***Trichoptera:*** *Calamoceratidae, Glossosomatidade, Philopotamidae, and Pschomylidae.*	8
Plecoptera:*Nemouridae.* ***Trichoptera:*** *Polycentropodidae.*	7
Crustacea. Trichoptera: *Hydrobioxidae, Hydroptilidae.*	6
Coleoptera: *Elmidae, Dryopidae.* ***Diptera:*** *Simuliidae, Tipulidae.* ***Ephemeroptera:*** *Euthyplociidae, Polymitarcidae.* ***Platyhelminthes. Trichoptera:*** *Helicopschidae, Hydropschidae.*	5
Arachnida:*Hydracarina.* ***Coleoptera:*** *Chrysomelidae, Curculionidae, Dixidae, Dolichopodidae.* ***Diptera:*** *Anthomyiidae, Ceratopogonidae, Chaoboridae, Dixidae, Dolichopodidae.* ***Empididae:*** *Limoniidae, Psychodidae, Stratiomyidae, and Tabanidae.* ***Ephemeroptera:*** *Bactidae, Caenidae.* ***Megaloptera:*** *Corydalidae, Stalidae.*	4
Annelida: *Hirudinea.* ***Coleoptera:*** *Dytiscidae, Gyrinidae, Helodidae, Hydrophilidae, and Noteridae.* ***Hemiptera:*** *Belostomatidae, Corixidae, Gerridae, Hydrometridae.* ***Mesoveliidae:*** *Naucoridae, Nepidae, Notonectidae, Pleidae, and Veliidae.* ***Mollusca.***	3
Diptera: *Chironomidae, Culucidae, Ephydridae, Muscidae, and Thaumaleidae.*	2
Annelida: *Oligochaeta.* ***Blattaria:*** *Blattidae.* ***Diptera:*** *Sciomyzidae, Syrphidae, and Rhagionidae.* ***Lepidoptera.***	1